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Process for producing anti-microbial effect with complex silver ions

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#### ABSTRACT OF THE DISCLOSURE

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6       Production of an anti-microbial effect in an alcohol or water based electrolyte  
7       is achieved by preparing silver materials that form complex ions other than  $\text{Ag}^+$ ,  $\text{Ag}^{2+}$ , or  $\text{Ag}^{3+}$ ,  
8       and which produce an anti-microbial effect that is greater than that produced by an equivalent  
9       amount of silver as  $\text{Ag}^+$ . Exemplary complex silver ions produced include  $\text{Ag}(\text{CN})_2^-$ ,  
10       $\text{AgCN}_{(aq)}^-(\text{ion pair})$ ,  $\text{Ag}(\text{NH}_3)_2^+$ ,  $\text{AgCl}_2^-$ , and  $\text{Ag}(\text{S}_2\text{O}_3)_2^{2-}$ . The silver materials must be prepared  
11      as powders or as solutions or suspensions containing the complex silver ions.

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AUSTRALIA

*Patents Act 1990*

# COMPLETE SPECIFICATION

## FOR A STANDARD PATENT ORIGINAL

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### TO BE COMPLETED BY APPLICANT

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Invention Title: PROCESS FOR PRODUCING ANTI-MICROBIAL  
EFFECT WITH COMPLEX SILVER IONS

Details of Associated Divisional Application No: 80551/94

The following statement is a full description of this invention, including the best method  
of performing it known to us:-

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## FIELD OF THE INVENTION

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The invention relates to methods of forming anti-microbial metal coatings, foils and powders which provide a sustained release of anti-microbial metal species when in contact with an alcohol or electrolyte.

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## BACKGROUND OF THE INVENTION

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The need for an effective anti-microbial coating is well established in the medical community. Physicians and surgeons using medical devices and appliances ranging from orthopaedic pins, plates and implants through to wound dressings and urinary catheters must constantly guard against infection. An inexpensive anti-microbial coating also finds application in medical devices used in consumer healthcare and personal hygiene products as well as in biomedical/biotechnical laboratory equipment. The term "medical device", as used herein and in the claims is meant to extend to all such products.

The anti-microbial effects of metallic ions such as Ag, Au, Pt, Pd, Ir (i.e. the noble metals), Cu, Sn, Sb, Bi and Zn are known (see Morton, H.E., *Pseudomonas in Disinfection, Sterilization and Preservation*, ed. S.S. Block, Lea and Febiger, 1977 and Grier, N., *Silver and Its Compounds in Disinfection, Sterilization and Preservation*, ed. S.S. Block, Lea and Febiger, 1977). Of the metallic ions with anti-microbial properties, silver is perhaps the best known due to its unusually good bioactivity at low concentrations. This phenomena is termed oligodynamic action. In modern medical practice both inorganic and organic soluble salts of silver are used to prevent and treat microbial infections. While these compounds are effective as soluble salts, they do not provide prolonged protection due to loss through removal or complexation of the free silver ions. They must be

1 reapplied at frequent intervals to overcome this problem. Reapplication is not always  
2 practical, especially where an in-dwelling or implanted medical device is involved.

3 Attempts have been made to slow the release of silver ions during treatment  
4 by creating silver containing complexes which have a lower level of solubility. For  
5 example, U.S. Patent 2,785,153 discloses colloidal silver protein for this purpose. Such  
6 compounds are usually formulated as creams. These compounds have not found wide  
7 applicability in the medical area due to their limited efficacy. The silver ion release rate  
8 is very slow. Furthermore, coatings from such compounds have been limited due to  
9 adhesion, abrasion resistance and shelf life problems.

10 The use of silver metal coatings for anti-microbial purposes has been  
11 suggested. For instance, see Deitch et al., Anti-microbial Agents and Chemotherapy, Vol.  
12 23(3), 1983, pp. 356 - 359 and Mackeen et al., Anti-microbial Agents and Chemotherapy,  
13 Vol. 31(1), 1987, pp. 93 - 99. However, it is generally accepted that such coatings alone  
14 do not provide the required level of efficacy, since diffusion of silver ions from the  
15 metallic surface is negligible.

16 A silver metal coating is produced by Spire Corporation, U.S.A. under the  
17 trade mark SPI-ARGENT. The coating is formed by an ion-beam assisted deposition  
18 (IBAD) coating process. The infection resistant coating is stated to be non-leaching in  
19 aqueous solutions as demonstrated by zone of inhibition tests, thus enforcing the belief that  
20 silver metal surfaces do not release anti-microbial amounts of silver ions.

21 Given the failure of metallic silver coatings to generate the required anti-  
22 microbial efficacy, other researchers have tried novel activation processes. One technique  
23 is to use electrical activation of metallic silver implants (see Marino et al., Journal of  
24 Biological Physics, Vol. 12, 1984, pp. 93 - 98). Electrical stimulation of metallic silver

1 is not always practical, especially for mobile patients. Attempts to overcome this problem  
2 include developing in situ electrical currents through galvanic action. Metal bands or  
3 layers of different metals are deposited on a device as thin film coatings. A galvanic cell  
4 is created when two metals in contact with each other are placed in an electrically  
5 conducting fluid. One metal layer acts as an anode, which dissolves into the electrolyte.  
6 The second metal acts as a cathode to drive the electrochemical cell. For example, in the  
7 case of alternating layers of Cu and Ag, the Cu is the anode, releasing Cu<sup>+</sup> ions into the  
8 electrolyte. The more noble of the metals, Ag, acts as the cathode, which does not ionize  
9 and does not go into solution to any large extent. An exemplary device of this nature is  
10 described in U.S. Patent 4,886,505 issued Dec. 12, 1989, to Haynes et al. The patent  
11 discloses sputtered coatings of two or more different metals with a switch affixed to one  
12 of the metals such that, when the switch is closed, metal ion release is achieved.

13 Previous work has shown that a film composed of thin laminates of  
14 alternating, different metals such as silver and copper can be made to dissolve if the  
15 surface is first etched. In this instance, the etching process creates a highly textured  
16 surface (see M. Tanemura and F. Okuyama, J. Vac. Sci. Technol., S, 1986, pp 2369-2372).  
17 However, the process of making such multilaminated films is time consuming and  
18 expensive.

19 Electrical activation of metallic coatings has not presented a suitable solution  
20 to the problem. It should be noted that galvanic action will occur only when an electrolyte  
21 is present and if an electrical connection between the two metals of the galvanic couple  
22 exists. Since galvanic corrosion occurs primarily at the metallic interface between the two  
23 metals, electrical contact is not sustained. Thus a continuous release of metal ions over  
24 an extended period of time is not probable. Also, galvanic action to release a metal such

1 as silver is difficult to achieve. As indicated above, the metal ions exhibiting the greatest  
2 anti-microbial effect are the noble metals, such as Ag, Au, Pt and Pd. There are few  
3 metals more noble than these to serve as cathode materials so as to drive the release of a  
4 noble metal such as Ag at the anode.

5 A second approach to activating the silver metal surface is to use heat or  
6 chemicals. U.S. Patents 4,476,590 and 4,615,705, issued to Scales et al. on October 16,  
7 1984 and October 7, 1986, respectively, disclose methods of activating silver surface  
8 coatings on endoprosthetic implants to render them bioerodible by heating at greater than  
9 180°C or by contacting with hydrogen peroxide. Such treatments are limited in terms of  
10 the substrate/devices which can be coated and activated.

11 There is still a need for an efficacious, inexpensive anti-microbial material  
12 having the following properties:

- 13 - sustained release of an anti-microbial agent at therapeutically active levels;
- 14 - applicable to a wide variety of devices and materials;
- 15 - useful shelf life; and
- 16 - low mammalian toxicity.

17 Metal coatings are typically produced as thin films by vapour deposition  
18 techniques such as sputtering. Thin films of metals, alloys, semiconductors and ceramics  
19 are widely used in the production of electronic components. These and other end uses  
20 require the thin films to be produced as dense, crystalline structures with minimal defects.  
21 The films are often annealed after deposition to enhance grain growth and recrystallization  
22 and produce stable properties. Techniques to deposit metal films are reviewed by R.F.  
23 Bunshah et al., "Deposition Technologies for Films and Coatings"; Noyes Publications,  
24 N.J., 1982 and by J.A. Thornton, "Influence of Apparatus Geometry and Deposition

1      Conditions on the Structure and Topography of Thick Sputtered Coatings", J. Vac. Sci.  
2      Technol., 11(4), 666-670, 1974.

3                U.S. Patent No. 4,325,776, issued April 20, 1982 to Menzel discloses a  
4      process for producing coarse or single crystal metal films from certain metals for use in  
5      integrated circuits. The metal film is formed by depositing on a cooled substrate (below -  
6      90°C) such that the metal layer is in an amorphous phase. The metal layer is then  
7      annealed by heating the substrate up to about room temperature. The end product is stated  
8      to have large grain diameter and great homogeneity, permitting higher current densities  
9      without electromigration failures.

10               Silver salts such as those of nitrate, proteins, acetate, lactate and citrate have  
11      been suggested for use in anti-microbial coatings for medical devices. Silver nitrate is  
12      used in burn wound dressings in many hospitals. These salts are known to have better  
13      anti-microbial efficacy than silver metal. The mechanism by which these compounds are  
14      effective is the instant ionization/dissociation to produce the Ag<sup>+</sup> ion. The availability of  
15      the Ag<sup>+</sup> ion is reduced significantly within or in contact with bodily fluids or tissues. Due  
16      to the high chloride content of such fluids, the silver is precipitated or tied up as insoluble  
17      silver chloride ( $K_{sp} = 1.7 \times 10^{-10}$ M). As a consequence, excessive amounts of silver must  
18      be present within any media containing precipitants (chiefly chloride) in order to produce  
19      the same efficacy from a silver salt as would be observed in water.

20               Nanocrystalline materials in the forms of powders, films and flakes are  
21      materials which are single-phase or multi-phase polycrystals, the grain size of which is in  
22      the order of a few (typically <20) nanometers in at least one dimension. Fine grain  
23      powders (particle size <5 microns) may be nanocrystalline, or more typically have grain  
24      sizes >20 nm. Nanocrystalline materials and fine powders may be prepared by a number

1 of modified gas condensation methods, wherein the material to be deposited is generated  
2 in the vapour phase, for example by evaporation or sputtering, and is transported into a  
3 relatively large volume in which the working gas atmosphere and temperature is controlled.  
4 Atoms of the material to be deposited collide with atoms of the working gas atmosphere,  
5 lose energy and are rapidly condensed from the vapour phase onto a cold substrate, such  
6 as a liquid nitrogen cooled finger. In principle, any method capable of producing very fine  
7 grain sized polycrystalline materials can be used to produce nanocrystalline materials.  
8 These methods include, for example, evaporation such as arc evaporation, electron beam  
9 vapor deposition, molecular beam epitaxy, ion beam, sputtering, magnetron sputtering and  
10 reactive sputtering (see for example, Froes, F.H. et al., "Nanocrystalline Metals for  
11 Structural Applications", JOM, 41 (1989), No. 6, pp 12 - 17; Birringer, Rainer et al.,  
12 "Nanocrystalline Materials - A First Report, Proceedings of JIMIS-4; and Gleiter, H.,  
13 "Materials with Ultrafine Microstructures: Retrospectives and Perspectives,  
14 NanoStructured Materials, Vol. 1, pp 1-19, 1992, and references cited therein).

15 **SUMMARY OF THE INVENTION**

16 The inventors set out to develop an anti-microbial metal coating. They  
17 discovered that, contrary to previous belief, it is possible to form metal coatings from an  
18 anti-microbial metal material by creating atomic disorder in the materials by vapour  
19 deposition under conditions which limit diffusion, that is which "freeze-in" the atomic  
20 disorder. The anti-microbial coatings so produced were found to provide sustained release  
21 of anti-microbial metal species into solution so as to produce an anti-microbial effect.

22 This basic discovery linking "atomic disorder" to enhanced solubility has  
23 broad application. The inventors have demonstrated that atomic disorder so as to produce

1       solubility can be created in other material forms, such as metal powders. The invention  
2       also has application beyond anti-microbial metals, encompassing any metal, metal alloy,  
3       or metal compound, including semiconductor or ceramic materials, from which sustained  
4       release of metal species into solution is desired. For instance, materials having enhanced  
5       or controlled metal dissolution find application in sensors, switches, fuses, electrodes, and  
6       batteries.

7                  The term "atomic disorder" as used herein includes high concentrations of:  
8       point defects in a crystal lattice, vacancies, line defects such as dislocations, interstitial  
9       atoms, amorphous regions, grain and sub grain boundaries and the like relative to its  
10      normal ordered crystalline state. Atomic disorder leads to irregularities in surface  
11      topography and inhomogenieties in the structure on a nanometre scale.

12                  By the term "normal ordered crystalline state" as used herein is meant the  
13      crystallinity normally found in bulk metal materials, alloys or compounds formed as cast,  
14      wrought or plated metal products. Such materials contain only low concentrations of such  
15      atomic defects as vacancies, grain boundaries and dislocations.

16                  The term "diffusion" as used herein implies diffusion of atoms and/or  
17      molecules on the surface or in the matrix of the material being formed.

18                  The terms "metal" or "metals" as used herein are meant to include one or  
19      more metals whether in the form of substantially pure metals, alloys or compounds such  
20      as oxides, nitrides, borides, sulphides, halides or hydrides.

21                  The invention, in a broad aspect extends to a method of forming a modified  
22      material containing one or more metals. The method comprises creating atomic disorder  
23      in the material under conditions which limit diffusion such that sufficient atomic disorder  
24      is retained in the material to provide release, preferably on a sustainable basis, of atoms,

1       ions, molecules or clusters of at least one of the metals into a solvent for the material.  
2       Clusters are known to be small groups of atoms, ions or the like, as described by R.P.  
3       Andres et al., "Research Opportunities on Clusters and Cluster-Assembled Materials", J.  
4       Mater. Res. Vol. 4, No. 3, 1989, P. 704.

5             Specific preferred embodiments of the invention demonstrate that atomic  
6       disorder may be created in metal powders or foils by cold working, and in metal coatings  
7       by depositing by vapour deposition at low substrate temperatures.

8             In another broad aspect, the invention provides a modified material  
9       comprising one or more metals in a form characterized by sufficient atomic disorder such  
10      that the material, in contact with a solvent for the material, releases atoms, ions, molecules  
11      or clusters containing at least one metal, preferably on a sustainable basis, at an enhanced  
12      rate relative to its normal ordered crystalline state.

13             In preferred embodiments of the invention, the modified material is a metal  
14      powder which has been mechanically worked or compressed, under cold working  
15      conditions, to create and retain atomic disorder.

16             The term "metal powder" as used herein is meant to include metal particles  
17      of a broad particle size, ranging from nanocrystalline powders to flakes.

18             The term "cold working" as used herein indicates that the material has been  
19      mechanically worked such as by milling, grinding, hammering, mortar and pestle or  
20      compressing, at temperatures lower than the recrystallization temperature of the material.  
21      This ensures that atomic disorder imparted through working is retained in the material.

22             In another preferred embodiment, the modified material is a metal coating  
23      formed on a substrate by vapour deposition techniques such as vacuum evaporation,  
24      sputtering, magnetron sputtering or ion plating. The material is formed under conditions

which limit diffusion during deposition and which limit annealing or recrystallization following deposition. The deposition conditions preferably used to produce atomic disorder in the coatings are outside the normal range of operating conditions used to produce defect free, dense, smooth films. Such normal practices are well known (see for example R.F. Bunshah et al., *supra*). Preferably the deposition is conducted at low substrate temperatures such that the ratio of the substrate temperature to the melting point of the metal or metal compound being deposited ( $T/T_m$ ) is maintained at less than about 0.5, more preferably at less than about 0.35, and most preferably at less than 0.30. In this ratio, the temperatures are in degrees Kelvin. The preferred ratio will vary from metal to metal and increases with alloy or impurity content. Other preferred deposition conditions to create atomic disorder include one or more of a higher than normal working gas pressure, a lower than normal angle of incidence of the coating flux and a higher than normal coating flux.

The temperature of deposition or cold working is not so low that substantial annealing or recrystallization will take place when the material is brought to room temperature or its intended temperature for use (ex. body temperature for anti-microbial materials). If the temperature differential between deposition and temperature of use ( $\Delta T$ ) is too great, annealing results, removing atomic disorder. This  $\Delta T$  will vary from metal to metal and with the deposition technique used. For example, with respect to silver, substrate temperatures of -20 to 200°C are preferred during physical vapour deposition.

Normal or ambient working gas pressure for depositing the usually required dense, smooth, defect free metal films vary according to the method of physical vapour deposition being used. In general, for sputtering, the normal working gas pressure is less than 10 Pa (Pascal) (75 mT (milliTorr)), for magnetron sputtering, less than 1.3Pa (10mT),

and for ion-plating less than 30Pa (200 mT). Normal ambient gas pressures for vacuum evaporation processes vary as follows: for e-beam or arc evaporation, from 0.0001 Pa (0.001 mT) to 0.001 Pa (0.01 mT); for gas scattering evaporation (pressure plating) and reactive arc evaporation, up to 30 Pa (200 mT), but typically less than 3 Pa (20mT).

- 5 Thus, in accordance with the method of the present invention, in addition to using low substrate temperatures to achieve atomic disorder, working (or ambient) gas pressures higher than these normal values may be used to increase the level of atomic disorder in the coating.

- Another condition discovered to have an effect on the level of atomic disorder  
10 in the coatings of the present invention is the angle of incidence of the coating flux during deposition. Normally to achieve dense, smooth coatings, this angle is maintained at about 90° +/- 15°. In accordance with the present invention, in addition to using low substrate temperatures during deposition to achieve atomic disorder, angles of incidence lower than about 75° may be used to increase the level of atomic disorder in the coating.

- 15 Yet another process parameter having an effect on the level of atomic disorder is the atom flux to the surface being coated. High deposition rates tend to increase atomic disorder, however, high deposition rates also tend to increase the coating temperature. Thus, there is an optimum deposition rate that depends on the deposition technique, the coating material and other process parameters.

- 20 To provide an anti-microbial material, the metals used in the coating or powder are those which have an anti-microbial effect, but which are biocompatible (non-toxic for the intended utility). Preferred metals include Ag, Au, Pt, Pd, Ir (i.e. the noble metals), Sn, Cu, Sb, Bi, and Zn, compounds of these metals or alloys containing one or more of these metals. Such metals are hereinafter referred to as "anti-microbial metals"). Most

1 preferred is Ag or its alloys and compounds. Anti-microbial materials in accordance with  
2 this invention preferably are formed with sufficient atomic disorder that atoms, ions,  
3 molecules or clusters of the anti-microbial material are released into an alcohol or water  
4 based electrolyte on a sustainable basis. The terms "sustainable basis" is used herein to  
5 differentiate, on the one hand from the release obtained from bulk metals, which release  
6 metal ions and the like at a rate and concentration which is too low to achieve an anti-  
7 microbial effect, and on the other hand from the release obtained from highly soluble salts  
8 such as silver nitrate, which release silver ions virtually instantly in contact with an alcohol  
9 or water based electrolyte. In contrast, the anti-microbial materials of the present invention  
10 release atoms, ions, molecules or clusters of the anti-microbial metal at a sufficient rate  
11 and concentration, over a sufficient time period to provide a useful anti-microbial effect.

12 The term "anti-microbial effect" as used herein means that atoms, ions,  
13 molecules or clusters of the anti-microbial metal are released into the electrolyte which the  
14 material contacts in concentrations sufficient to inhibit bacterial growth in the vicinity of  
15 the material. The most common method of measuring anti-microbial effect is by  
16 measuring the zone of inhibition (ZOI) created when the material is placed on a bacterial  
17 lawn. A relatively small or no ZOI (ex. less than 1 mm) indicates a non-useful anti-  
18 microbial effect, while a larger ZOI (ex. greater than 5 mm) indicates a highly useful anti-  
19 microbial effect. One procedure for a ZOI test is set out in the Examples which follow.

20 The invention extends to devices such as medical devices formed from,  
21 incorporating, carrying or coated with the anti-microbial powders or coatings. The anti-  
22 microbial coating may be directly deposited by vapour deposition onto such medical  
23 devices as catheters, sutures, implants, burn dressings and the like. An adhesion layer,  
24 such as tantalum, may be applied between the device and the anti-microbial coating.

1 Adhesion may also be enhanced by methods known in the art, for example etching the  
2 substrate or forming a mixed interface between the substrate and the coating by  
3 simultaneous sputtering and etching. Anti-microbial powders may be incorporated into  
4 creams, polymers, ceramics, paints, or other matrices, by techniques well known in the art.

5 In a further broad aspect of the invention, modified materials are prepared  
6 as composite metal coatings containing atomic disorder. In this case, the coating of the  
7 one or more metals or compounds to be released into solution constitutes a matrix  
8 containing atoms or molecules of a different material. The presence of different atoms or  
9 molecules results in atomic disorder in the metal matrix, for instance due to different sized  
10 atoms. The different atoms or molecules may be one or more second metals, metal alloys  
11 or metal compounds which are co- or sequentially deposited with the first metal or metals  
12 to be released. Alternatively the different atoms or molecules may be absorbed or trapped  
13 from the working gas atmosphere during reactive vapour deposition. The degree of atomic  
14 disorder, and thus solubility, achieved by the inclusion of the different atoms or molecules  
15 varies, depending on the materials. In order to retain and enhance the atomic disorder in  
16 the composite material, one or more of the above-described vapour deposition conditions,  
17 namely low substrate temperature, high working gas pressure, low angle of incidence and  
18 high coating flux, may be used in combination with the inclusion of different atoms or  
19 molecules.

20 Preferred composite materials for anti-microbial purposes are formed by  
21 including atoms or molecules containing oxygen, nitrogen, hydrogen, boron, sulphur or  
22 halogens in the working gas atmosphere while depositing the anti-microbial metal. These  
23 atoms or molecules are incorporated in the coating either by being absorbed or trapped in  
24 the film, or by reacting with the metal being deposited. Both of these mechanisms during

1 deposition are hereinafter referred to as "reactive deposition". Gases containing these  
2 elements, for example oxygen, hydrogen, and water vapour, may be provided continuously  
3 or may be pulsed for sequential deposition.

4 Anti-microbial composite materials are also preferably prepared by co- or  
5 sequentially depositing an anti-microbial metal with one or more inert biocompatible  
6 metals selected from Ta, Ti, Nb, Zn, V, Hf, Mo, Si, and Al. Alternatively, the composite  
7 materials may be formed by co-, sequentially or reactively depositing one or more of the  
8 anti-microbial metals as the oxides, carbides, nitrides, borides, sulphides or halides of these  
9 metals and/or the oxides, carbides, nitrides, borides, sulphides or halides of the inert  
10 metals. Particularly preferred composites contain oxides of silver and/or gold, alone or  
11 together with one or more oxides of Ta, Ti, Zn and Nb.

12 The invention also extends to a method of activating or further enhancing  
13 the anti-microbial effect of anti-microbial materials formed with atomic disorder. Thus,  
14 anti-microbial materials made in accordance with the present invention may be irradiated  
15 to further enhance the anti-microbial effect. However, it is also possible to irradiate  
16 materials initially formed with a level of atomic disorder which is insufficient to produce  
17 an anti-microbial effect, such that the irradiated material has an acceptable anti-microbial  
18 effect. The process of activation comprises irradiating the material with a low linear  
19 energy transfer form of radiation such as beta or x-rays, but most preferably gamma rays.  
20 A dose greater than 1 Mrad is preferred. The anti-microbial material is preferably oriented  
21 substantially perpendicular to the incoming radiation. The level of activation may be  
22 further enhanced by irradiating the material adjacent to a dielectric material such as oxides  
23 of Ta, Al and Ti, but most preferably silicon oxide.

1           The invention also extends to the preparation of anti-microbial silver  
2        materials which form complex silver ions other than  $\text{Ag}^+$ ,  $\text{Ag}^{2+}$  and  $\text{Ag}^{3+}$ , in contact with  
3        an alcohol or a water based electrolyte. The complex silver ions are found to have a  
4        surprisingly greater anti-microbial efficacy than does the  $\text{Ag}^+$  ion released from the silver  
5        salts of the prior art. Exemplary complex silver ions include  $\text{Ag}(\text{CN})_2$ ,  $\text{AgCN}_{(aq)}$ (ion pair),  
6         $\text{Ag}(\text{NH}_3)_2^+$ ,  $\text{AgCl}_2^-$ ,  $\text{Ag}(\text{OH})_2$ ,  $\text{Ag}_2(\text{OH})_3$ ,  $\text{Ag}_3(\text{OH})_4$  and  $\text{Ag}(\text{S}_2\text{O}_3)_2$ . Silver coatings,  
7        powders, flakes and foils prepared with atomic disorder in accordance with the present  
8        invention are exemplary of silver materials which release complex silver ions having anti-  
9        microbial efficacy. Alternatively the silver materials may be prepared as solutions,  
10        ointments, paints or suspensions containing the complex silver ions. Such silver materials  
11        have wide application, for example as coatings for medical devices, in topical anti-  
12        microbial compositions, in anti-fouling paints or coatings and as coatings for anti-microbial  
13        filters.

14           Thus, in accordance with a broad aspect of the invention, there is provided  
15        a method of producing an anti-microbial effect in an alcohol or a water based electrolyte  
16        comprising, preparing a silver material such that it forms complex silver ions other than  
17         $\text{Ag}^+$ ,  $\text{Ag}^{2+}$  and  $\text{Ag}^{3+}$  in an amount so as to produce an anti-microbial effect in contact with  
18        an alcohol or water-based electrolyte that is greater than that produced by an equivalent  
19        amount of silver as  $\text{Ag}^+$ ; and bringing the silver material in contact with the surface,  
20        alcohol or electrolyte to be treated so as to cause the release of the complex silver ions.

21           The invention further extends to fine grain anti-microbial materials in a fine  
22        powder, film or flake form, comprising one or more anti-microbial metals or alloys or  
23        compounds thereof, having a grain size less than 200 nm, in a fine powder, flake or film  
24        form, characterized by sufficient atomic disorder such that the material, in contact with an

1 alcohol or a water based electrolyte, provides a sustained release of the atoms, ions,  
2 molecules or clusters of at least one anti-microbial metal into the alcohol or water based  
3 electrolyte at a concentration sufficient to provide a localized anti-microbial effect.

4                 The anti-microbial material may be prepared by introducing the atomic  
5 disorder into a pre-formed fine grain or nanocrystalline (<20 nm) powder, flakes or films  
6 of one or more of the anti-microbial metals by mechanical working, for example by  
7 compressing the material, under cold working conditions. Alternatively, the atomic  
8 disorder may be created during the synthesis of fine grain or nanocrystalline materials  
9 (films, flakes or powders) by vapour deposition techniques in which the anti-microbial  
10 metal is co-, sequentially or reactively deposited in a matrix with atoms or molecules of  
11 a different material under conditions such that atomic disorder is created and retained in  
12 the matrix. The different material (or dopant) is selected from inert biocompatible metals,  
13 oxygen, nitrogen, hydrogen, boron, sulphur, and halogens, and oxides, nitrides, carbides,  
14 borides, sulphides and halides of either of both of an anti-microbial metal or a  
15 biocompatible metal. Preferred biocompatible metals include Ta, Ti, Nb, B, Hf, Zn, Mo,  
16 Si and Al. These different materials may be included with the anti-microbial metal in the  
17 same or separate target, for example a target of Ag and/or silver oxides, which may further  
18 contain, for example, Ta or tantalum oxides. Alternatively, the different material may be  
19 introduced from the working gas atmosphere during vapour deposition, for example by  
20 sputtering or reactive sputtering in an atmosphere containing atoms or molecules of the  
21 different material such as oxygen.

22                 The anti-microbial form of silver material prepared in accordance with the  
23 process of the present invention has been physically characterized and has been found to  
24 have the following novel characteristics:

8                    Each of these physical characteristics, with perhaps the exception of grain  
9 size, is believed to be the result of the presence of atomic disorder in the material. The  
10 characteristics are of assistance in identifying and distinguishing the silver materials of the  
11 present invention from prior art materials or materials in their normal ordered crystalline  
12 state. The preferred novel anti-microbial silver materials have been characterized, for  
13 example by XRD, XPS and SIMS analysis, as comprising substantially pure silver metal,  
14 when deposited in an inert atmosphere such as argon. However, when the working gas  
15 atmosphere contains oxygen, the materials comprise a matrix of substantially pure silver  
16 metal and one or both of, silver oxide and atoms or molecules of trapped or absorbed  
17 oxygen. A further distinguishing feature of the materials of the present invention is the  
18 presence of growth twins in the grain structure, visible from TEM analysis.

**BRIEF DESCRIPTION OF THE DRAWINGS**

20 Figure 1 is a TEM micrograph of a sputter deposited silver coating in  
21 accordance with the invention, illustrating grain size and growth twin defects.

22 Figure 2 is a TEM micrograph of the film of Figure 1 after annealing.  
23 showing larger grain size and the presence of annealing twins.

1                   **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

2                   As above stated, the present invention has application beyond anti-microbial  
3                   materials. However, the invention is disclosed herein with anti-microbial metals, which  
4                   are illustrative of utility for other metals, metal alloys and metal compounds. Preferred  
5                   metals include Al and Si, and the metal elements from the following groups of the periodic  
6                   table: IIIB, IVA, VB, VIB, VIIIB, IB, IIB, IIIA, IVA, and VA (excluding As) in  
7                   the periods 4, 5 and 6, (see Periodic Table as published in Merck Index 10th Ed., 1983,  
8                   Merck and Co. Inc., Rahway, N.J., Martha Windholz). Different metals will have varying  
9                   degrees of solubility. However, the creation and retention of atomic disorder in accordance  
10                  with this invention results in enhanced solubility (release) of the metal as ions, atoms,  
11                  molecules or clusters into an appropriate solvent i.e. a solvent for the particular material,  
12                  typically a polar solvent, over the solubility of the material in its normal ordered crystalline  
13                  state.

14                  The medical devices formed from, incorporating, carrying or coated with the  
15                  anti-microbial material of this invention generally come into contact with an alcohol or  
16                  water based electrolyte including a body fluid (for example blood, urine or saliva) or body  
17                  tissue (for example skin, muscle or bone) for any period of time such that microorganism  
18                  growth on the device surface is possible. The term "alcohol or water based electrolyte"  
19                  also includes alcohol or water based gels. In most cases the devices are medical devices  
20                  such as catheters, implants, tracheal tubes, orthopaedic pins, insulin pumps, wound  
21                  closures, drains, dressings, shunts, connectors, prosthetic devices, pacemaker leads, needles,  
22                  surgical instruments, dental prostheses, ventilator tubes and the like. However, it should  
23                  be understood that the invention is not limited to such devices and may extend to other  
24                  devices useful in consumer healthcare, such as sterile packaging, clothing and footwear,

1 personal hygiene products such as diapers and sanitary pads, in biomedical or biotechnical  
2 laboratory equipment, such as tables, enclosures and wall coverings, and the like. The  
3 term "medical device" as used herein and in the claims is intended to extend broadly to  
4 all such devices.

5 The device may be made of any suitable material, for example metals,  
6 including steel, aluminum and its alloys, latex, nylon, silicone, polyester, glass, ceramic,  
7 paper, cloth and other plastics and rubbers. For use as an in-dwelling medical device, the  
8 device will be made of a bioinert material. The device may take on any shape dictated by  
9 its utility, ranging from flat sheets to discs, rods and hollow tubes. The device may be  
10 rigid or flexible, a factor again dictated by its intended use.

11 Anti-Microbial Coatings

12 The anti-microbial coating in accordance with this invention is deposited as  
13 a thin metallic film on one or more surfaces of a medical device by vapour deposition  
14 techniques. Physical vapour techniques, which are well known in the art, all deposit the  
15 metal from the vapour, generally atom by atom, onto a substrate surface. The techniques  
16 include vacuum or arc evaporation, sputtering, magnetron sputtering and ion plating. The  
17 deposition is conducted in a manner to create atomic disorder in the coating as defined  
18 hereinabove. Various conditions responsible for producing atomic disorder are useful.  
19 These conditions are generally avoided in thin film deposition techniques where the object  
20 is to create a defect free, smooth and dense film (see for example J.A. Thornton, supra).  
21 While such conditions have been investigated in the art, they have not heretofore been  
22 linked to enhanced solubility of the coatings so-produced.

1                   The preferred conditions which are used to create atomic disorder during the  
2 deposition process include:

3                   - a low substrate temperature, that is maintaining the surface to be coated  
4 at a temperature such that the ratio of the substrate temperature to the melting point of the  
5 metal (in degrees Kelvin) is less than about 0.5, more preferably less than about 0.35 and  
6 most preferably less than about 0.3; and optionally one or both of:

7                   - a higher than normal working (or ambient) gas pressure, i.e. for vacuum  
8 evaporation: e-beam or arc evaporation, greater than 0.001 Pa (0.01 mT), gas scattering  
9 evaporation (pressure plating) or reactive arc evaporation, greater than 3 Pa (20 mT); for  
10 sputtering: greater than 10 Pa (75 mT); for magnetron sputtering: greater than about 1.3  
11 Pa (10 mT); and for ion plating: greater than about 30 Pa (200 mT); and

12                   - maintaining the angle of incidence of the coating flux on the surface to be  
13 coated at less than about 75°, and preferably less than about 30°

14                   The metals used in the coating are those known to have an anti-microbial  
15 effect. For most medical devices, the metal must also be biocompatible. Preferred metals  
16 include the noble metals Ag, Au, Pt, Pd, and Ir as well as Sn, Cu, Sb, Bi, and Zn or alloys  
17 or compounds of these metals or other metals. Most preferred is Ag or Au, or alloys or  
18 compounds of one or more of these metals.

19                   The coating is formed as a thin film on at least a part of the surface of the  
20 medical device. The film has a thickness no greater than that needed to provide release  
21 of metal ions on a sustainable basis over a suitable period of time. In that respect, the  
22 thickness will vary with the particular metal in the coating (which varies the solubility and  
23 abrasion resistance), and with the degree of atomic disorder in (and thus the solubility of)  
24 the coating. The thickness will be thin enough that the coating does not interfere with the

1 dimensional tolerances or flexibility of the device for its intended utility. Typically,  
2 thicknesses of less than 1 micron have been found to provide sufficient sustained anti-  
3 microbial activity. Increased thicknesses may be used depending on the degree of metal  
4 ion release needed over a period of time. Thicknesses greater than 10 microns are more  
5 expensive to produce and normally should not be needed.

6 The anti-microbial effect of the coating is achieved when the device is  
7 brought into contact with an alcohol or a water based electrolyte such as, a body fluid or  
8 body tissue, thus releasing metal ions, atoms, molecules or clusters. The concentration of  
9 the metal which is needed to produce an anti-microbial effect will vary from metal to  
10 metal. Generally, anti-microbial effect is achieved in body fluids such as plasma, serum  
11 or urine at concentrations less than about 0.5 - 1.5  $\mu\text{g}/\text{ml}$ .

12 The ability to achieve release of metal atoms, ions, molecules or clusters on  
13 a sustainable basis from a coating is dictated by a number of factors, including coating  
14 characteristics such as composition, structure, solubility and thickness, and the nature of  
15 the environment in which the device is used. As the level of atomic disorder is increased,  
16 the amount of metal ions released per unit time increases. For instance, a silver metal film  
17 deposited by magnetron sputtering at  $T/T_m < 0.5$  and a working gas pressure of about 0.9  
18 Pa (7 mTorr) releases approximately 1/3 of the silver ions that a film deposited under  
19 similar conditions, but at 4 Pa (30 mTorr), will release over 10 days. Films that are  
20 created with an intermediate structure (ex. lower pressure, lower angle of incidence etc.)  
21 have Ag release values intermediate to these values as determined by bioassays. This then  
22 provides a method for producing controlled release metallic coatings in accordance with  
23 this invention. Slow release coatings are prepared such that the degree of disorder is low  
24 while fast release coatings are prepared such that the degree of disorder is high.

1           For continuous, uniform coatings, the time required for total dissolution will  
2       be a function of film thickness and the nature of the environment to which they are  
3       exposed. The relationship in respect of thickness is approximately linear, i.e. a two fold  
4       increase in film thickness will result in about a two fold increase in longevity.

5           It is also possible to control the metal release from a coating by forming a  
6       thin film coating with a modulated structure. For instance, a coating deposited by  
7       magnetron sputtering such that the working gas pressure was low (ex. 2 Pa (15 mTorr))  
8       for 50% of the deposition time and high (ex. 4 Pa (30 mTorr)) for the remaining time, has  
9       a rapid initial release of metal ions, followed by a longer period of slow release. This type  
10      of coating is extremely effective on devices such as urinary catheters for which an initial  
11      rapid release is required to achieve immediate anti-microbial concentrations followed by  
12      a lower release rate to sustain the concentration of metal ions over a period of weeks.

13           The substrate temperature used during vapour deposition should not be so  
14      low that annealing or recrystallization of the coating takes place as the coating warms to  
15      ambient temperatures or the temperatures at which it is to be used (ex. body temperature).  
16      This allowable  $\Delta T$ , that the temperature differential between the substrate temperature  
17      during deposition and the ultimate temperature of use, will vary from metal to metal. For  
18      the most preferred metals of Ag and Au, preferred substrate temperatures of -20 to 200°C  
19      , more preferably -10°C to 100°C are used.

20           Atomic disorder may also be achieved, in accordance with the present  
21      invention, by preparing composite metal materials, that is materials which contain one or  
22      more anti-microbial metals in a metal matrix which includes atoms or molecules different  
23      from the anti-microbial metals.

1               Our technique for preparing composite material is to co- or sequentially  
2 deposit the anti-microbial metal(s) with one or more other inert, biocompatible metals  
3 selected from Ta, Ti, Nb, Zn, V, Hf, Mo, Si, Al and alloys of these metals or other metal  
4 elements, typically other transition metals. Such inert metals have a different atomic radii  
5 from that of the anti-microbial metals, which results in atomic disorder during deposition.  
6               Alloys of this kind can also serve to reduce atomic diffusion and thus stabilize the  
7 disordered structure. Thin film deposition equipment with multiple targets for the  
8 placement of each of the anti-microbial and inert metals is preferably utilized. When  
9 layers are sequentially deposited the layer(s) of the inert metal(s) should be discontinuous,  
10 for example as islands within the anti-microbial metal matrix. The final ratio of the anti-  
11 microbial metal(s) to inert metal(s) should be greater than about 0.2. The most preferable  
12 inert metals are Ti, Ta, Zn and Nb. It is also possible to form the anti-microbial coating  
13 from oxides, carbides, nitrides, sulphides, borides, halides or hydrides of one or more of  
14 the anti-microbial metals and/or one or more of the inert metals to achieve the desired  
15 atomic disorder.

16               Another composite material within the scope of the present invention is  
17 formed by reactively co- or sequentially depositing, by physical vapour techniques, a  
18 reacted material into the thin film of the anti-microbial metal(s). The reacted material is  
19 an oxide, nitride, carbide, boride, sulphide, hydride or halide of the anti-microbial and/or  
20 inert metal, formed in situ by injecting the appropriate reactants, or gases containing same,  
21 (ex. air, oxygen, water, nitrogen, hydrogen, boron, sulphur, halogens) into the deposition  
22 chamber. Atoms or molecules of these gases may also become absorbed or trapped in the  
23 metal film to create atomic disorder. The reactant may be continuously supplied during  
24 deposition for codeposition or it may be pulsed to provide for sequential deposition. The

- 1 final ratio of anti-microbial metal(s) to reaction product should be greater than about 0.2.
- 2 Air, oxygen, nitrogen and hydrogen are particularly preferred reactants.

3                 The above deposition techniques to prepare composite coatings may be used  
4 with or without the conditions of lower substrate temperatures, high working gas pressures  
5 and low angles of incidence previously discussed. One or more of these conditions is  
6 preferred to retain and enhance the amount of atomic disorder created in the coating.

7                 It may be advantageous, prior to depositing an anti-microbial in accordance  
8 with the present invention, to provide an adhesion layer on the device to be coated, as is  
9 known in the art. For instance, for a latex device, a layer of Ti, Ta or Nb may be first  
10 deposited to enhance adhesion of the subsequently deposited anti-microbial coating.

11                 Anti-Microbial Powders

12                 Anti-microbial powders, including nanocrystalline powders and powders  
13 made from rapidly solidified flakes or foils, can be formed with atomic disorder so as to  
14 enhance solubility. The powders either as pure metals, metal alloys or compounds such  
15 as metal oxides or metal salts, can be mechanically worked or compressed to impart  
16 atomic disorder. This mechanically imparted disorder is conducted under conditions of  
17 low temperature (i.e. temperatures less than the temperature of recrystallization of the  
18 material) to ensure that annealing or recrystallization does not take place. The temperature  
19 varies between metals and increases with alloy or impurity content

20                 Anti-microbial powders produced in accordance with this invention may be  
21 used in a variety of forms, for instance in topical creams, paints or adherent coatings.  
22 Alternatively, the powder may be incorporated into a polymeric, ceramic or metallic matrix  
23 to be used as a material for medical devices or coatings therefor.

1        Fine Grain or Nanocrystalline Materials of Anti-Microbial Metals

2              Methods of forming fine grain or nanocrystalline materials from the vapour  
3        phase are well known and documented in the literature. For instance, nanocrystalline  
4        materials may be formed by a modified standard inert-gas condensation technique. The  
5        material to be deposited is evaporated from an electrically heated boat or crucible into an  
6        inert gas atmosphere such as argon or helium with a pressure of about 5 to 7 Torr. The  
7        temperature of the boat has to be high enough to obtain a substantial vapour pressure of  
8        the material of interest. For metals, a temperature about 100°C above the melting point  
9        of the metal will typically provide an adequate vapour pressure. Due to interatomic  
10      collisions with the working gas atmosphere atoms, the evaporated atoms of the material  
11      lose their kinetic energy and condense onto a cold finger or substrate held at about 77 K  
12      (liquid nitrogen cooled) in the form of a loose powder or friable flakes or film, the grain  
13      size of which is less than about 20 nm. With respect to powders or flakes, a high vacuum  
14      (less than  $5 \times 10^{-6}$  Pa) is restored and the powder or flakes are stripped off from the cold  
15      finger and collected in a cold trap.

16              Fine grain materials are produced analogously in gas condensation/vapour  
17        deposition processes, as is known in the art. This is typically achieved by altering the cold  
18        finger or substrate temperature and the gas pressure to allow the particle to coarsen to the  
19        desired size which is preferably under 5000 nm.

20              Fine powders/nanocrystalline powders of anti-microbial metals prepared in  
21        accordance with the known prior art processes have been tested and found not to have  
22        sufficient anti-microbial efficacy. In order to introduce atomic disorder into the materials  
23        at a level which is sufficient to produce an anti-microbial effect, the anti-microbial metal,  
24        alloy or compound to be deposited is co-, sequentially or reactively deposited in a matrix

1 with atoms or molecules of a different material (dopant) under conditions such that atomic  
2 disorder is created and retained in the matrix. The different material is selected from inert  
3 biocompatible metals, such as Ta, Ti, Nb, B, Hf, Zn, Mo, Si and Al, most preferably Ta,  
4 Ti and Nb. Alternatively the different material is an oxide, nitride, carbide, boride,  
5 sulphide or halide of either or both of an anti-microbial metal or of the biocompatible  
6 metal. A further alternative is to introduce the different material from the working gas  
7 atmosphere, either by reactive deposition or by absorbing or trapping atoms or molecules  
8 from the working gas into the matrix. Working gas atmospheres containing oxygen,  
9 nitrogen, hydrogen, boron, sulphur and halogens may be used. Working gas atmospheres  
10 including oxygen are most preferred, such that the matrix of anti-microbial metal includes  
11 either or both of trapped oxygen and oxides of the anti-microbial metal.

12 A further technique for forming anti-microbial powders of the present  
13 invention is to form coatings containing atomic disorder in the manner set out above onto  
14 an inert, preferably biocompatible, particulate material such as talc, bentonite, cornstarch  
15 or ceramics such as alumina. The particles may be coated by physical vapour deposition  
16 techniques under conditions to create atomic disorder, as set forth above in respect of the  
17 anti-microbial metal coatings. Alternatively, the powders can be coated by adapting a  
18 vapour deposition process, for instance by passing a vapour of the anti-microbial material  
19 through a fixed porous bed of the powders, by fluidizing the powder bed in the anti-  
20 microbial metal vapour phase, or by letting the powder fall through a vapour of the anti-  
21 microbial material. In all cases, the powder could be cooled and/or the working gas  
22 atmosphere could be altered to include a different material (ex. oxygen), in order to  
23 produce the desired degree of atomic disorder.

1        Activation of Anti-Microbial Materials

2              Irradiation of anti-microbial materials (powders, nanocrystalline powders,  
3              foils, coatings or composite coatings of anti-microbial metals) which contain atomic  
4              disorder formed by any of the above-described procedures, will further activate or enhance  
5              the anti-microbial effect. Thus, even materials having a low level of atomic disorder may  
6              be activated to an anti-microbial level.

7              Irradiation is performed with any low linear energy transfer form of  
8              radiation, including beta, gamma and x-rays. Gamma radiation at a dose of 1 Mrad or  
9              greater is preferred. Since gamma radiation is an acceptable method of sterilization of  
10             medical devices, activation and sterilization may be achieved simultaneously through the  
11             irradiation process of the present invention.

12             The irradiation step is preferably conducted such that the anti-microbial  
13             material being irradiated is oriented generally perpendicular to the incoming radiation  
14             (rather than parallel). A further enhancement of the anti-microbial effect can be achieved  
15             by conducting the irradiation step with a dielectric material adjacent to, or preferably  
16             sandwiched around the anti-microbial material. Exemplary dielectrics include oxides of  
17             Si, Ti, Ta and Al. Silicon oxide surfaces are preferred. It is believed that the dielectric  
18             material provides forward scattering of electrons into the anti-microbial coating.

19             Without being bound by the same it is believed that the irradiation step is  
20             causing one or more of the following changes in the anti-microbial material:

- 21             1)       creating further atomic disorder, such as point defects;
- 22             2)       enhancing oxygen adsorption/chemisorption to the surface of the anti-microbial  
23             material;
- 24             3)       activating trapped dopant atoms or molecules such as oxygen to O<sup>+</sup> or O<sub>2</sub><sup>-</sup>; and

1       4) creating broken or dangling bonds at the surface.  
2       With respect to the second and third proposed mechanisms, it is possible that oxygen  
3       adsorption/chemisorption and/or activation creates a super saturated concentration of O<sub>2</sub>,  
4       O<sup>+</sup> or O<sub>2</sub><sup>-</sup> species in or on the anti-microbial metal surface, which results in the more rapid  
5       dissolution of the anti-microbial metal or species thereof into an aqueous environment  
6       through the generation of various chemical species of the anti-microbial metal, including  
7       oxides and hydroxides.

8       Silver Materials Forming Complex Silver Ions

9                  In accordance with the invention, silver materials are prepared which form  
10       complex silver ions other than Ag<sup>+</sup>, Ag<sup>2+</sup> and Ag<sup>3+</sup>, when the material is contacted with an  
11       alcohol or a water based electrolyte. Exemplary complex silver ions shown to demonstrate  
12       an anti-microbial effect include Ag(CN)<sub>2</sub><sup>-</sup>, AgCN<sub>(aq)</sub><sup>(ion pair)</sup>, Ag(NH<sub>3</sub>)<sub>2</sub><sup>+</sup>, AgCl<sub>2</sub><sup>-</sup>, Ag(OH)<sub>2</sub><sup>-</sup>,  
13       Ag<sub>2</sub>(OH)<sub>3</sub><sup>-</sup>, Ag<sub>3</sub>(OH)<sub>4</sub><sup>-</sup> and Ag(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub><sup>3-</sup>. These silver materials forming complex silver ions  
14       have wide application, for instance, as anti-microbial coatings for medical devices, as anti-  
15       microbial powders for medical or pharmaceutical use, as anti-fouling paints, coatings or  
16       compositions, anti-microbial coatings for filters and the like.

17                  It should be understood that the phrase "silver materials which form  
18       complex silver ions other than Ag<sup>+</sup>, Ag<sup>2+</sup> and Ag<sup>3+</sup>" as used herein and in the claims is not  
19       intended to exclude silver materials which form one or more of Ag<sup>+</sup>, Ag<sup>2+</sup> and Ag<sup>3+</sup> ions  
20       in addition to the complex silver ions when the material contacts an alcohol or a water  
21       based electrolyte. The notation Ag<sup>+</sup>, Ag<sup>2+</sup> and Ag<sup>3+</sup>- refers to these ions in solution and  
22       includes solvated forms. The term complex silver ions as used herein and in the claims

1 is not intended to include silver ions stabilized with strong oxidizing agents, such as  
2 persulphate and periodate, to prevent the reduction of the silver ions.

3 The anti-microbial coatings, powders and foils of the present invention,  
4 when created with atomic disorder as above described, are exemplary of silver materials  
5 which form complex silver ions other than  $\text{Ag}^+$  so as to cause an anti-microbial effect. It  
6 is believed that the complex silver ions which may be formed when such silver materials  
7 contact an alcohol or water based electrolyte, are one or more of the negative ions  
8  $\text{Ag(OH)}_1^-$ ,  $\text{Ag}_2(\text{OH})_2^-$  and  $\text{Ag}_3(\text{OH})_4^-$ .

9 Silver materials which form complex silver ions may also be prepared by  
10 bringing a silver metal, compound or salt into an environment containing excessive  
11 amounts of a cationic, anionic or neutral species with which it is desired to complex silver.  
12 For example, the negative complex silver ion  $\text{AgCl}_2^-$  can be generated by placing a silver  
13 salt such as  $\text{AgNO}_3$  in an aqueous medium with an elevated concentration of the  $\text{Cl}^-$  ion.  
14  $\text{AgNO}_3/\text{NaCl}$  or  $\text{AgCl}/\text{NaCl}$  mixtures, solutions or suspensions can form the  $\text{AgCl}_2^-$  ion.  
15 This  $\text{AgCl}_2^-$  ion may also be generated with mixtures of silver powder with  $\text{NaCl}$ .  
16 Preferably the silver powder is one which is prepared in accordance with the present  
17 invention so as to contain atomic disorder, but bulk silver may also be activated in this  
18 manner. Bulk silver powder, fine grain (<140 nm) and nanocrystalline (<20 nm) powders  
19 may be used. Similarly, the ion  $\text{Ag}(\text{NH}_3)_2^+$  can be formed in aqueous solution by adding  
20 silver salts to excess ammonium hydroxide. The ion  $\text{Ag(S}_2\text{O}_3)_2^+$  may be formed in  
21 aqueous solution by adding silver salts to excess sodium thiosulphate. The ion  $\text{Ag}(\text{CN})_2^-$   
22 may be formed in aqueous solution by adding excess potassium cyanide to silver cyanide.

23 The silver materials forming complex silver ions may be formulated for use  
24 in many forms, including for example, powders, suspensions, solutions, ointments or

1       coatings. For instance, a pharmaceutical composition to generate the AgCl<sub>x</sub> ion can be  
2       formulated as a mixture of the salts AgNO<sub>3</sub>/NaCl or as a mixture of NaCl with a silver  
3       powder, preferably one containing atomic disorder. These mixtures of the silver material  
4       might be pre-formulated as a solution, suspension or ointment with a sterile aqueous or  
5       saline solution and pharmaceutically acceptable carriers, diluents, excipients and the like.  
6       Alternatively the silver material might be provided as the mixtures of silver powder/NaCl  
7       salt or AgNO<sub>3</sub>/NaCl, for later formulation by the end user.

8       Physical/Chemical Characteristics of Anti-Microbial Silver Material

9           The modified metal materials formed in accordance with the present  
10      invention so as to contain atomic disorder which leads to enhanced release of the metal  
11      species have novel physical characteristics when compared with materials in their normal  
12      ordered crystalline state. Silver materials made in accordance with the present invention  
13      have been characterized as having the following novel characteristics:

- 14           - a positive rest potential, E<sub>rest</sub>, for example, when measured against a SCE  
15      reference electrode in a 1 M KOH solution;
- 16           - preferably a ratio of temperature of recrystallization to melting temperature  
17      less than 0.33, and most preferably less than 0.30;
- 18           - preferably a temperature of recrystallization less than about 140 °C; and
- 19           - preferably a grain size less than about 200nm, more preferably less than  
20      140nm and most preferably less than 90nm.

21           Analysis of the silver materials by XRD, XPS and SIMS techniques  
22      confirms the chemical nature and content of the film as silver metal, and in the event that  
23      the material is formed with oxygen in the working gas atmosphere, one or both of silver

1 oxide and trapped oxygen. TEM analysis reveals growth twins in the silver material,  
2 which are converted to annealed twins when the materials are annealed above the  
3 temperature of recrystallization.

4 The invention is further illustrated by the following non-limiting examples.

5 Example 1

6 A medical suture material size 2/0, polyester braid was coated by magnetron  
7 sputtering 20.3 cm diameter (8 in.) from planar silver and copper magnetron cathodes to  
8 form an Ag-Cu-alloy on the surface to a thickness of 0.45 microns, using either argon gas  
9 working pressures of 0.9 Pa (7 mTorr) or 4 Pa (30 mT) at 0.5 KW power and a T/Tm ratio  
10 of less than 0.5. The total mass flow of gas was 700 sccm (standard cubic centimeters per  
11 minute).

12 The anti-microbial effect of the coatings was tested by a zone of inhibition  
13 test. Basal medium Eagle (BME) with Earle's salts and L-glutamine was modified with  
14 calf/serum (10%) and 1.5 % agar prior to being dispensed (15 ml) into Petri dishes. The  
15 agar containing Petri plates were allowed to surface dry prior to being inoculated with a  
16 lawn of *Staphylococcus aureus* ATCC# 25923. The inoculant was prepared from Bacurol  
17 Discs (Disco, M.) which were reconstituted as per the manufacturer's directions.  
18 Immediately after inoculation, the materials or coatings to be tested were placed on the  
19 surface of the agar. The dishes were incubated for 24 h at 37°C. After this incubation  
20 period, the zone of inhibition was measured and a corrected zone of inhibition was  
21 calculated (corrected zone of inhibition = zone of inhibition - diameter of the test material  
22 in contact with the agar).

1           The results showed no zone of inhibition on the uncoated suture, a zone of  
2           less than 0.5 mm around the suture coated at 0.9 Pa (7 mTorr) and a zone of 13 mm  
3           around the suture coated at 4 Pa (30 mTorr). Clearly the suture coated in accordance with  
4           the present invention exhibits a much more pronounced and effective anti-microbial effect.

5           Example 2

6           This example is included to illustrate the surface structures which are  
7           obtained when silver metal is deposited on silicon wafers using a magnetron sputtering  
8           facility and different working gas pressures and angles of incidence (i.e. the angle between  
9           the path of the sputtered atoms and the substrate). All other conditions were as follows:  
10          target was a 20.3 cm diameter planar silver magnetron cathode; power was 0.1 kW;  
11          deposition rate was 200 Å/min; ratio of temperature of substrate (wafer) to melting point  
12          of silver (1234°K), T/T<sub>m</sub> was less than 0.3. Argon gas pressures of 0.9 Pa (7 mTorr) (a  
13          normal working pressure for metal coatings) and 4 Pa (30 mTorr) were used with a total  
14          mass flow of 700 sccm. Angles of incidence at each of these pressures were 90° (normal  
15          incidence), 50° and 10°. The coatings had a thickness of about 0.5 microns.

16          The resulting surfaces were viewed by scanning electron microscope. As  
17          argon gas pressure increased from 0.9 Pa (7 mTorr) to 4 Pa (30 mTorr) the grain size  
18          decreased and void volume increased significantly. When the angle of incidence was  
19          decreased, the grain size decreased and the grain boundaries became more distinct. At 0.9  
20          Pa (7 mTorr) argon pressure and an angle of incidence of 10°, there were indications of  
21          some voids between the grains. The angle of incidence had a greater effect on the surface  
22          topography when the gas pressure was increased to 4 Pa (30 mTorr). At 90°, the grain size  
23          varied from 60 - 150 nm and many of the grains were separated by intergrain void spaces

1 which were 15 - 30 nm wide. When the angle of incidence was decreased to 50°, the grain  
2 size decreased to 30 - 90 nm and the void volume increased substantially. At 10°, the  
3 grain size was reduced to about 10 - 60 nm and void volumes were increased again.

4 The observed nanometre scale changes in surface morphology and  
5 topography are indications of atomic disorder in the silver metal. While not being bound  
6 by the same, it is believed that such atomic disorder results in an increase in the chemical  
7 activity due to increased internal stresses and surface roughness created by mismatched  
8 atoms. It is believed that the increased chemical activity is responsible for the increased  
9 level of solubility of the coatings when in contact with an electrolyte such as body fluid.

10 The anti-microbial effect of the coatings was evaluated using the zone of  
11 inhibition test as set out in Example 1. Each coated silicon wafer was placed on an  
12 individual plate. The results were compared to the zones of inhibition achieved when solid  
13 silver (i.e. greater than 99% silver) sheets, wires or membranes were tested. The results  
14 are summarized in Table 1. It is evident that the pure silver devices and the silver  
15 sputtered coating at 0.9 Pa (7 mTorr) do not produce any biological effect. However, the  
16 coatings deposited at a higher than normal working gas pressure, 4 Pa (30 mTorr),  
17 demonstrated an anti-microbial effect, as denoted by the substantial zones of inhibition  
18 around the discs. Decreasing the angle of incidence had the greatest effect on anti-  
19 microbial activity when combined with the higher gas pressures.

1

Table I

2 Anti-microbial effects of various silver and silver coated samples as determined using *Staphylococcus*  
 3 *aureus*

4	5 Sample	6 Percent Silver	7 Angle of Deposition	8 Working Gas Pressure (mTorr)	9 Corrected Zone of Inhibition (mm)
10	Silver Sheet- rolled	99+	-	-	<0.5
11	Silver wire (.0045")	99+	-	-	<0.5
12	Silver membrane- cast	99+	-	-	<0.5
13	Sputtered thin film	99+	normal (90°)	0.9 (7)	<0.5
14	Sputtered thin film	99+	50°	0.9 (7)	<0.5
15	Sputtered thin film	99+	10°	0.9 (7)	<0.5
16	Sputtered thin film	99+	normal (90°)	4 (30)	6.3
17	Sputtered thin film	99+	50°	4 (30)	10
18	Sputtered thin film	99+	10	4 (30)	10

36 Example 3

37 Silicon wafers were coated by magnetron sputtering using 20.3 cm diameter  
 38 planar silver and copper magnetron cathodes to produce an alloy of Ag and Cu (80:20) at  
 39 normal incidence at working gas pressures of 0.9 Pa (7 mTorr) and 4 Pa (30 mTorr), all  
 40 other conditions being identical to those set out in Example 2. As in Example 2, when the  
 41 coatings were viewed by SEM, the coatings formed at high working gas pressure had

1 smaller grain sizes and larger void volumes than did the coatings formed at the lower  
2 working gas pressures.

3 Coatings which were similarly formed as a 50:50 Ag/Cu alloy were tested  
4 for anti-microbial activity with the zone of inhibition test set out in Example 1. The  
5 results are summarized in Table 2. Coatings deposited at low working gas pressure (0.9  
6 Pa (7 mTorr)) showed minimal zones of inhibition, while the coatings deposited at high  
7 working gas pressure (4 Pa (30 mTorr)) produced larger zones of inhibition, indicative of  
8 anti-microbial activity.

9  
10 Table 2  
11 The anti-microbial effect of various sputter deposited silver-copper alloys as determined using *Staphylococcus*  
*aureus*

12	Sample	Percent Silver	Angle of Deposition (°)	Working Gas Pressure Pa (mTorr)	Corrected Zone of Inhibition (mm)
13	1	50	normal (90°)	1.0 (7.5)	<0.5
14	2	50	normal (90°)	4 (30)	16
15	3	50	10	4 (30)	19
16					
17					
18					
19					
20					
21					
22					
23					
24	Example 4				

25 A coating in accordance with the present invention was tested to determine  
26 the concentration of silver ions released into solution over time. One cm<sup>2</sup> silicon wafer  
27 discs were coated with silver as set forth in Example 2 at 0.9 Pa (7 mTorr) and 4 Pa (30  
28 mTorr) and normal incidence to a thickness of 5000 Å. Using the method of Nickel et

1 al., Eur. J. Clin. Microbiol., 4(2), 213-218, 1985, a sterile synthetic urine was prepared and  
2 dispensed into test tubes (3.5 ml). The coated discs were placed into each test tubes and  
3 incubated for various times at 37°C. After various periods of time, the discs were removed  
4 and the Ag content of the filtered synthetic urine was determined using neutron activation  
5 analysis.

6 The results are set forth in Table 3. The table shows the comparative  
7 amounts of Ag released over time from coatings deposited on discs at 0.9 Pa (7 mTorr)  
8 or 4 Pa (30 mTorr). The coatings deposited at high pressure were more soluble than those  
9 deposited at low pressure. It should be noted that this test is a static test. Thus, silver  
10 levels build up over time, which would not be the case in body fluid where there is  
11 constant turn over.

12 Table 3

13 Concentration of silver in synthetic urine as a function of exposure time

14 Silver Concentration  $\mu\text{g}/\text{ml}$

15 Exposure Time (Days)	16 Working Argon gas pressure 0.9 Pa (7mTorr)	17 Working argon gas pressure 4 Pa (30mTorr)
20 0	ND1	ND
21 1	0.89	1.94
22 3	1.89	2.36
23 10	8.14	23.06

24 Note: Films were deposited at normal incidence (90°)  
25 1 - ND (non detectable) <0.46  $\mu\text{g}/\text{ml}$

### 1 Example 5

This example is included to illustrate coatings in accordance with the present invention formed from another noble metal, Pd. The coatings were formed on silicon wafers as set forth in Example 2, to a thickness of 5000 Å<sup>0</sup>, using 0.9 Pa (7 mTorr) or 4 Pa (30 mTorr) working gas pressures and angles of incidence of 90° and 10°. The coated discs were evaluated for anti-microbial activity by the zone of inhibition test substantially as set forth in Example 1. The coated discs were placed coating side up such that the agar formed a 1 mm surface coating over the discs. The medium was allowed to solidify and surface dry, after which the bacterial lawn was spread over the surface. The dishes were incubated at 37°C for 24 h. The amount of growth was then visually analyzed.

The results are set forth in Table 4. At high working gas pressures, the biological activity of the coating was much greater than that of coatings deposited at low pressure. Changing the angle of incidence (decreasing) improved the anti-microbial effect of the coating to a greater extent when the gas pressure was low than when it was high.

Table 4

16 Surface Control of *Staphylococcus aureus* by Sputter Deposited Palladium metal

Sample	Sputtering Pressure Pa (mTorr)	Angle of Deposition	Anti-microbial Control
1	0.9 (7)	90°(normal incidence)	More than 90% of surface covered by bacterial growth
2	0.9 (7)	10°(grazing incidence)	20-40% of surface covered by bacterial growth
3	4 (30)	90°(normal incidence)	Less than 10% surface covered by bacterial growth

1      Example 6

2            This example is included to illustrate the effect of silver deposition  
3        temperature on the anti-microbial activity of the coating. Silver metal was deposited on  
4        2.5 cm sections of a latex Foley catheter using a magnetron sputtering facility. Operating  
5        conditions were as follows; the deposition rate was 200 Å<sup>0</sup> per minute; the power was 0.1  
6        kW; the target was a 20.3 cm diameter planar silver magnetron cathode; the argon working  
7        gas pressure was 4 Pa (30mTorr); the total mass flow was 700 sccm; and the ratio of  
8        temperature of substrate to melting point of the coating metal silver, T/T<sub>m</sub> was 0.30 or  
9        0.38. In this example the angles of incidence were variable since the substrate was round  
10       and rough. That is the angles of incidence varied around the circumference and, on a finer  
11       scale, across the sides and tops of the numerous surface features. The anti-microbial effect  
12       was tested by a zone of inhibition test as outlined in Example 1.

13            The results showed corrected zones of inhibition of 0.5 and 16 mm around  
14        the tubing coated at T/T<sub>m</sub> values of 0.38 and 0.30 respectively. The sections of Foley  
15        catheter coated at the lower T/T<sub>m</sub> value were more efficacious than those coated at higher  
16        T/T<sub>m</sub> value.

17      Example 7

18            This example is included to demonstrate an anti-microbial coating formed  
19        by DC magnetron sputtering on a commercial catheter. A teflon coated latex Foley  
20        catheter was coated by DC magnetron sputtering 99.99% pure silver on the surface using  
21        the conditions listed in Table 5. The working gases used were commercial Ar and 99/1  
22        wt% Ar/O<sub>2</sub>.

1           The anti-microbial effect of the coating was tested by a zone of inhibition  
2       test. Mueller Hinton agar was dispensed into Petri dishes. The agar plates were allowed  
3       to surface dry prior to being inoculated with a lawn of *Staphylococcus aureus* ATCC#  
4       25923. The inoculant was prepared from Bactrol Discs (Difco, M.) which were  
5       reconstituted as per the manufacturer's directions. Immediately after inoculation, the  
6       coated materials to be tested were placed on the surface of the agar. The dishes were  
7       incubated for 24 hr. at 37°C. After this incubation period, the zone of inhibition was  
8       measured and a corrected zone of inhibition was calculated (corrected zone of inhibition  
9       = zone of inhibition - diameter of the test material in contact with the agar).

10          The results showed no zone of inhibition for the uncoated samples and a  
11       corrected zone of less than 1 mm for catheters sputtered in commercial argon at a working  
12       gas pressure of 0.7 Pa (5 mT). A corrected zone of inhibition of 11 mm was reported for  
13       the catheters sputtered in the 99/1 wt% Ar/O<sub>2</sub> using a working gas pressure of 5.3 Pa (40  
14       mT). XRD analysis showed that the coating sputtered in 1% oxygen was a crystalline Ag  
15       film. This structure clearly caused an improved anti-microbial effect for the coated  
16       catheters.

**Table 5**  
**Conditions of DC Magnetron Sputtering Used for Anti-Microbial Coatings**

	Samples Sputtered in Commercial Argon	Samples Sputtered in 99/1 wt% Ar/O <sub>x</sub>
Power	0.1 kW	0.5 kW
Target	20.3 cm dia Ag	20.3 cm dia Ag
Argon Pressure	0.7 Pa (5 m Torr)	Ar/O <sub>x</sub> Pressure: 5.3 Pa (40 m Torr)
Total Mass Flow	700 sccm	700 sccm
Initial Substrate Temperature	20°C	20°C
Cathode/Anode Distance	40 mm	100 mm
Film Thickness	2500 Å	3000 Å

#### Example 8

This example demonstrates silver coatings formed by arc evaporation, gas scattering evaporation (pressure plating) and reactive arc evaporation. Evaporation of 99.99% silver was performed onto silicon or alumina wafers at an initial substrate temperature of about 21°C, using the parameters as follows:

9 Bias: -100 V

20. Current: 20 Amp-hrs

i : Angle of incidence: 90°

Working Gas Pressure: 0.001 Pa (0.01 mT) (arc), 3.5 Pa (26 mT) Ar/H<sub>2</sub> 96:4 (gas scattering evaporation), and 3.5 Pa (26 mT) O<sub>2</sub> (reactive arc evaporation)

No corrected ZOI was observed for wafers coated at vacuum (arc). Pressure plating with a working gas atmosphere containing Ar and 4 % hydrogen produced a 6 mm ZOI, while a working gas atmosphere of pure oxygen (reactive arc) produced an 8 mm ZOI. Film thicknesses of about 4000 Angstroms were produced. The results indicate that

1 the presence of gases such as hydrogen and/or oxygen in the arc evaporation atmosphere  
2 cause the coatings to have improved anti-microbial efficacy.

3 Example 9

4 This example is included to illustrate composite materials to produce anti-  
5 microbial effects. A set of coatings were produced by RF magnetron sputtering zinc oxide  
6 onto silicon wafers as outlined below. The zinc oxide coatings showed no zone of  
7 inhibition.

8 Coatings of Ag and ZnO were deposited to a total thickness of 3300  
9 Angstroms by sequentially sputtering layers of Ag with layers of ZnO, according to the  
10 conditions below, in a 75/25 wt% ratio. The coatings were demonstrated to have no zone  
11 of inhibition when the zinc oxide layers were about 100 Angstroms thick. However, films  
12 consisting of islands of very thin to discontinuous layers of ZnO (less than 50 Angstroms)  
13 in an Ag matrix (ie. a composite film) had a 8 mm corrected zone of inhibition.

14 The conditions used to deposit ZnO were as follows:

15 Target 20.3 cm dia Zno; Working gas = argon; Working gas pressure = 4 Pa (30 mT);  
16 Cathode-Anode distance: 40 mm; Initial Substrate Temperature: 21°C; Power: RF  
17 magnetron, 0.5 kW.

18 The conditions used to deposit the Ag were as follows:

19 Target 20.3 cm dia Ag; Working gas = argon; Working gas pressure = 4 Pa (30 mT);  
20 Cathode-Anode distance = 40 mm; Initial Substrate Temperature = 21°C; Power = DC  
21 magnetron, 0.1 kW.

1      Example 10

2                This example demonstrates the effect of cold working and annealing silver  
3          and gold powders on the anti-microbial efficacy demonstrated by a standard zone of  
4          inhibition test. Cold working of such powders results in a defective surface structure  
5          containing atomic disorder which favours the release of ions causing anti-microbial  
6          activity. The anti-microbial effect of this defective structure can be removed by annealing.

7                Nanocrystalline silver powder (crystal size about 30 nm) was sprinkled onto  
8          adhesive tape and tested. A zone of inhibition of 5 mm was obtained, using the method  
9          set forth in Example 7. A 0.3g pellet of the nanocrystalline Ag powder was pressed at  
10        275,700 kPa (kiloPascal) (40,000 psi). The pellet produced a 9 mm zone of inhibition  
11        when tested for anti-microbial activity. Nanocyrstalline silver powder was mechanically  
12        worked in a ball mill for 30 sec. The resulting powder was tested for anti-microbial  
13        activity, both by sprinkling the worked powder on adhesive tape and applying to the plates,  
14        and by pressing the powder into a pellet at the above conditions and placing the pellet on  
15        the plates. The zones of inhibition observed were 7 and 11 mm respectively. A pellet that  
16        had been pressed from the worked powder was annealed at 500°C for 1 hour under vacuum  
17        conditions. A reduced zone of inhibition of 3 mm was observed for the annealed pellet.

18               These results demonstrate that nanocrystalline silver powder, while having  
19        a small anti-microbial effect on its own, has an improved anti-microbial effect by  
20        introducing atomic disorder by mechanical working of the powder in a ball mill or by  
21        pressing it into a pellet. The anti-microbial effect was significantly decreased by annealing  
22        at 500°C. Thus, conditions of mechanical working should not include or be followed by  
23        conditions such as high temperature, which allow diffusion. Cold mechanical working

1 conditions are preferred to limit diffusion, for example by working at room temperature  
2 or by grinding or milling in liquid nitrogen.

3 Silver powder, 1 micron particle size, was tested in a manner similar to  
4 above. The Ag powder sprinkled onto adhesive tape and tested for a zone of inhibition.  
5 No zone of inhibition was observed. The powder was worked in a ball mill for 30 seconds  
6 and sprinkled onto adhesive tape. A 6 mm zone of inhibition was observed around the  
7 powder on the tape. When the Ag powder (as is or after mechanical working in the ball  
8 mill) was pressed into a 0.3 g pellet using 275,700 kPa (40,000 psi), zones of inhibition  
9 of 5 and 6 mm respectively were observed. A pellet which was formed from the ball  
10 milled powder and which was annealed at 500°C for 1 hour had significantly reduced anti-  
11 microbial activity. Initially the pellet had some activity (4.5 mm zone of inhibition) but  
12 after the pellet was tested a second time, no zone of inhibition was observed. A control  
13 pellet which had not been annealed continued to give a zone of inhibition greater than 4  
14 mm even after 14 repeats of the test. This demonstrates that an annealing step, following  
15 mechanical working, limits the sustainable release of the anti-microbial silver species from  
16 the powders.

17 Nanocrystalline gold (20 nm crystals), supplied as a powder, was tested for  
18 anti-microbial effect by sprinkling the powder onto adhesive tape and using the zone of  
19 inhibition test. No zone of inhibition was recorded for the nanocrystalline gold powder.  
20 The gold powder was pressed into a 0.2 g pellet using 275,700 kPa (40,000 psi). A 10  
21 mm zone of inhibition was observed. When the pressed pellets were subsequently vacuum  
22 annealed at 500°C for 1 hour and the zone of inhibition was found to be 0 mm.

23 The results showed that solubility and thus the anti-microbial efficacy of  
24 gold powders can be improved by a mechanical working process such as pressing a

1 nanocrystalline material into a pellet. The anti-microbial activity can be removed by  
2 annealing. Cold working is preferred.

3 Other gold powders including a 2-5 micron and a 250 micron particle size  
4 powder did not demonstrate an anti-microbial effect under the above mechanical working  
5 conditions. It is believed that the small grain size of the nanocrystalline gold powder was  
6 an important cofactor which, with the mechanical working, produced the desired anti-  
7 microbial effect.

8 Example 11

9 This example is included to demonstrate a composite anti-microbial coating  
10 formed by reactive sputtering (another example of composite films). Example 7  
11 demonstrates that an anti-microbial coating of silver can be obtained by sputtering in argon  
12 and 1% oxygen (0.5 kW, 5.3 Pa (40 mTorr), 100 mm anode/cathode distance, and 20°C -  
13 produced a zone of inhibition of 11 mm).

14 When a working gas of argon and 20 wt% oxygen was used to sputter anti-  
15 microbial coatings under the conditions listed below, the zones of inhibition ranged from  
16 6 to 12 mm. This indicates that the provision of a reactive atmosphere during vapour  
17 deposition has the result of producing an anti-microbial film over a wide range of  
18 deposition process parameters.

19... Table 6 - Sputtering Conditions

20	Target	20.3 cm dia, 99.99% Ag
21	Working Gas:	80/20 wt% Ar/O <sub>2</sub>
22	Working Gas Pressure:	0.3 to 6.7 Pa (2.5 to 50 mTorr)
23	Total Mass Gas Flow:	700 sccm
24	Power:	0.1 to 2.5 kW
25	Substrate Temperature:	-5 to 20°C
26	Anode/Cathode Distance	40 to 100 mm
27	Base Pressure:	less than 5 x 10 <sup>-4</sup> Pa (4 x 10 <sup>-6</sup> Torr)

1        Example 12

2              This example demonstrates that the coatings of this invention have an anti-  
3        microbial effect against a broad spectrum of bacteria.

4              A total of 171 different bacterial samples encompassing 18 genera and 55  
5        species were provided by the Provincial Laboratory of Public Health for Northern Alberta.  
6        These samples had been quick frozen in 20% skim milk and stored at -70°C for periods  
7        ranging from several months to several years. Fastidious organisms which were unlikely  
8        to grow under conditions used in standard Kirby-Bauer susceptibility testing were not used.

9              Each frozen sample was scraped with a sterile cotton swab to inoculate a  
10      blood agar plate (BAP). The plates were incubated overnight at 35°C. The following  
11      morning isolated colonies were subcultured onto fresh BAPs and incubated at 35°C  
12      overnight. The next day, the organisms were subjected to Kirby-Bauer susceptibility  
13      testing as described below.

14             Four to five colonies (more if colonies were small) of the same  
15      morphological type were selected from each BAP subculture and inoculated into individual  
16      tubes containing approximately 5 mL of tryptic soy broth (TSB). The broths were  
17      incubated at 35°C for approximately 2 to 3 hours. At this time, the turbidity of most of  
18      the broth cultures either equalled or exceeded that of a 0.5 McFarland standard. The more  
19      turbid samples were diluted with sterile saline to obtain a turbidity visually comparable to  
20      that of the standard. To aid in the visual assessment of turbidity, tubes were read against  
21      a white background with contrasting black line.

22             A small number of the organisms (*Streptococcus* and *Corynebacterium*) did  
23      not grow well in TSB. The turbidity of these broths, after incubation, was less than that

1 of the 0.5 McFarland standard. Additional colonies from the BAP subcultures were  
2 inoculated to these tubes to increase the turbidity to approximate that of the standard.

3 Within 15 minutes of adjusting the turbidity of the bacterial suspensions a  
4 sterile cotton swab was dipped into each broth. Excess fluid was removed by rotating the  
5 swab against the rim of the tube. The inoculum was applied to a Mueller Hinton (MH)  
6 agar plate by streaking the swab evenly in three directions over the entire agar surface.  
7 Three 1 cm x 1 cm silver coated silica wafer squares were applied to each MH plate and  
8 the plates were inverted and incubated overnight at 35°C. The coatings had been sputtered  
9 under the following conditions, which through XRD analysis were shown to be silver/silver  
10 oxide composite films:

11:	Target:	20.3 cm dia, 99.99% Ag
12:	Working gas:	80/20 wt % Ar/O <sub>2</sub>
13:	Working gas pressure:	5.3 Pa (40 mT)
14:	Total Mass Gas Flow:	700 sccm
15:	Power:	0.1 kW
16:	Temperature of Deposition	20°C
17:	Base pressure	2.7 X 10 <sup>-4</sup> Pa (2 x 10 <sup>-6</sup> Torr)
18:	Cathode/anode distance	40 mm

19 BAP cultures of control organisms were provided by the Provincial  
20 Laboratory and included: *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa*  
21 ATCC 27853; *Escherichia coli* ATCC 25922; and *Enterococcus faecalis* ATCC 29212 to  
22 check the quality of the MH agar. These cultures were treated in a like manner to the test  
23 organisms except that standard antibiotic discs rather than silver coated wafers were  
24 applied to the bacterial lawns on the MH agar. These organisms demonstrated that the MH  
25 agar was suitable for standard ZOI tests.

26 After 16 to 18 hours of incubation at 35°C zones of inhibition around the  
27 silver wafers or antibiotic discs were measured to the nearest mm. Corrected zones were

calculated by subtracting the size of the wafer (1 cm) from the size of the total zone.

2 Representative zone of inhibition results are shown in Table 7.

**Table 7**  
**The Sensitivity of a Broad Range of Microorganisms to Silver<sup>+</sup> Coated Silicon Wafers**

Organism	Source	Corrected Zone of Inhibition (mm)
<i>Staphylococcus epidermidis</i> RC-455	blood	10
<i>Bacillus licheniformis</i> R-2138	tibia	6
<i>Corynebacterium</i> sp R-594	leg	10
<i>Listeria monocytogenes</i> R-590	blood	5
<i>Enterococcus faecalis</i> SR-113	bone	5
<i>Streptococcus bovis</i> SR-62	blood	10
<i>Escherichia coli</i> R-1878	urine	11
<i>Klebsiella ozonae</i> R-308/90	abdomen	10
<i>Enterobacter cloacae</i> R-1682	unknown	8
<i>Proteus vulgaris</i> 3781	urine	4
<i>Providencia stuartii</i> U-3179	urine	8
<i>Citrobacter freundii</i> U-3122/90	urine	7
<i>Salmonella typhimurium</i> ER-1154	urine	6
<i>Serratia marcescens</i> R-850	spurum	6
<i>Pseudomonas aeruginosa</i> U-3027	urine	10
<i>Xanthomonas maliphila</i> 90-10B	unknown	9
<i>Aeromonas caviae</i> R-1211	wound	5
<i>Branhamella catarrhalis</i> R-2681	unknown	12
Silver deposition*		

### 6 Example 13

47 This example demonstrates the use of tantalum as an adhesive layer for  
48 coatings of this invention. Tantalum is well known as a material which, in the form of an  
49 interlayer, improves adhesion of thin films to substrates. In this example test sections

1 including a group of stainless steel (316) (1 x 1 cm) and silicon (1.7 X 0.9 cm) coupons  
2 and sections of latex tubing (5 cm) were cleaned in ethanol and then half of the test  
3 sections were coated (by sputtering) with a thin layer (approx. 100 Angstroms) of Ta  
4 before an anti-microbial silver film was deposited on them. The second group of the test  
5 sections were only coated with the anti-microbial Ag film. Coating conditions are listed  
6 below. While all test sections had similar anti-microbial activity, the Ta coated test  
7 sections had much better adhesion properties than did the untreated test sections. Adhesion  
8 properties were determined using ASTM method D3359-87, a standard test method for  
9 measuring adhesion.

## **10 Sputtering Conditions**

11	Target:	20.3 cm dia, 99.99% Ta
12	Working Gas:	99/1 wt% Ar/O <sub>2</sub>
13	Working Gas Pressure:	1.3 Pa (10 mTorr)
14	Total Mass Gas Flow:	700 sccm
15	Power:	0.5 kW
16	Cathode/Anode Distance:	100 mm
17	Substrate Temperature:	20°C
18	Target:	20.3 cm dia, 99.99% Ag
19	Working Gas:	99/1 wt% Ar/O <sub>2</sub>
20	Working Gas Pressure:	5.3 Pa (40 mTorr)
21	Total Mass Gas Flow:	700 sccm
22	Power:	0.5 kW
23	Cathode/Anode Distance:	100 mm
24	Substrate Temperature:	20°C

#### **Example 14**

DC magnetron sputtering was used to deposit silver from a 20.3 cm dia,  
99.98% pure cathode onto silicon and alumina wafers with commercial argon moisturized  
with water as the working gas at a total mass gas flow of 700 sccm. The argon was  
moisturized by passing it through two flasks containing 3 litres of room temperature water  
and one empty flask set up with glass wool to absorb any free liquid before the gas entered  
the sputtering unit.

The conditions of sputtering and the results of the standard zone of inhibition test performed on the sputtered silver films are shown below. Silver films which normally had no anti-microbial properties when deposited using argon that had not been treated with water yielded a corrected zone of inhibition of up to 8 mm when sputtered using a argon/water vapour mixture as the working gas.

**Table 8**  
**Conditions used for DC Magnetron Sputtering of Anti-Microbial Coatings**

	Working Gas	Working Gas Pressure Pa (mTorr)	Power	Substrate Temperature	Anode/Cathode Distance	Corrected ZOI
14	Commercial Argon	1.3 (10)	0.5kW	-10°C	100 mm	0 mm
15	Ar passed through H <sub>2</sub> O	1.3 (10)	0.5kW	-10°C	100 mm	8 mm

### Example 15

This example is included to illustrate the method of activating coatings with radiation, in accordance with another aspect of the present invention.

A series of  $1.9 \times 0.7$  cm silicon wafers were coated with  $3000 \text{ \AA}$  coatings of silver metal using DC magnetron sputtering under the following conditions:

## Sputtering Conditions

24..	Target	20.3 cm dia, 99.99% Ag
25..	Working Gas	99/1 wt% Ar/O <sub>2</sub>
26..	Working Gas pressure	5.3 Pa (40 mTorr)
27..	Total Mass Gas Flow	700 sccm
28..	Power	0.5 kW
29..	Substrate Temperature	21°C
30	Anode/Cathode Distance	100 mm

31 The coated wafers were divided into 4 groups and irradiated with varying doses of gamma  
32 radiation - 0, 1, 2 and 4 megarad doses - from a  $^{60}\text{Co}$  source at Isomedix Inc., Morton  
33 Grove, Ill., U.S.A. The samples were placed generally perpendicular to the incoming

1 radiation. After irradiation, the samples were tested for biological activity (anti-microbial  
2 effect) using a standard zone of inhibition test on Mueller Hinton Agar (Difco, Mi) with  
3 *S. aureus* (ATCC #25923), as set out in previous examples. The results are summarized  
4 in Table 9.

5 **Table 9**  
6 Effects of Gamma Radiation on Biological Activity of Anti-Microbial Coatings

7	Gamma Radiation Dose (megarads)	Corrected Zone of Inhibition (mm)
8	0	11
9	1	14
10	2	17
11	4	20

12 The results generally show a log dose response relationship between the  
13 radiation dose and the observed biological response to the wafers. This illustrates that the  
14 gamma radiation has further activated the coatings of the present invention to enhance the  
15 anti-microbial effect.

16 The experiment was repeated with the anti-microbial films being oriented  
17 generally parallel to the incoming radiation. This orientation substantially reduced the  
18 level of activation of the anti-microbial coatings, such that no increase in the zone of  
19 inhibition was observed relative to controls which had not been irradiated.

20 Example 16

21 This example is included to illustrate activation of the anti-microbial  
22 coatings in accordance with the present invention with gamma radiation using a dielectric  
23 material adjacent to the material during irradiation.

24 A number of 2.5 cm x 2.5 cm pieces of high density polyethylene mesh  
25 (such as used in burn wound dressings) were sputter coated with silver metal under the

1 same conditions as set forth in Example 15 with the exception that the power was 0.1 kW.  
2 The coated mesh was then irradiated (perpendicular orientation) as set forth in Example  
3 15 at 4 megarads. The biological activity was then tested, as set out in Example 15.  
4 Control mesh samples (silver coated, no irradiation) gave a 10mm ZOI(corrected), while  
5 the irradiated samples gave a 14 mm ZOI(corrected).

6 Further samples of the coated mesh were irradiated while sandwiched  
7 between two 2.5 cm x 2.5 cm silicon wafers having a 1000 Å thermally grown oxide layer,  
8 as supplied by the Alberta Microelectronics Centre, Edmonton, Alberta. This mesh sample  
9 was tested for biological activity and was found to produce a 26 mm ZOI(corrected).  
10 Without being bound by the same, it is believed that the silicon wafers provide a source  
11 of electrons which are forward scattered to the anti-microbial coatings, further enhancing  
12 the anti-microbial effect

13 Bulk silver sheet metal was tested to determine whether it could be activated  
14 to produce an anti-microbial effect by gamma irradiation. The bulk silver sheet metal  
15 samples were annealed at 140°C for 90 minutes in air and then irradiated with a 4 megarad  
16 dose. The samples were tested for biological activity, but no ZOI was produced. This  
17 result appears to indicate that bulk silver, in its normal ordered crystalline state, has too  
18 few atomic defects to be activated in accordance with the process of the present invention.

19 Example 17.

20 This example is included to illustrate that anti-microbial coatings containing  
21 atomic disorder at a level that is insufficient to produce an anti-microbial effect can be  
22 further activated by gamma irradiation, in accordance with the present invention.

1           Silver films were sputtered onto silicon wafers, as set forth in Example 15.  
2           except that the gas pressure was reduced from 5.3 Pa (40 mTorr) to 0.7 Pa (5 mTorr),  
3           resulting in less atomic disorder in the coatings. The silver films were then irradiated with  
4           a 4 Mrad dose of gamma radiation, as in Example 15. The irradiated and control films  
5           (not irradiated) were tested for biological activity. The control films produced only 1 mm  
6           ZOI(corrected), while the irradiated coatings produced 10 mm ZOI(corrected). This result  
7           demonstrates that anti-microbial materials prepared under conditions such that they contain  
8           atomic disorder at a level insufficient to produce an anti-microbial effect can be activated  
9           so as to be anti-microbial by irradiating with a source of gamma radiation.

10          Example 18

11          This example is included to demonstrate the generation of silver complex  
12          ions which are distinct from the Ag<sup>+</sup> ion and which are highly efficacious in generating  
13          an anti-microbial effect. The example provides comparative diffusion and zone of  
14          inhibition (ZOI) data for various silver solutions.

15          Solutions were prepared to generate 10,000 ppm Ag as AgNO<sub>3</sub>, Ag(NH<sub>3</sub>)<sub>2</sub><sup>+</sup>,  
16          Ag(CN)<sub>2</sub><sup>-</sup>, Ag(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub><sup>3-</sup> and Ag(protein).

17          The silver solutions were prepared as follows:

- 18       1) Ag(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub><sup>3-</sup> - 2.66 g of AgCl were dissolved in 150 ml of deionized water.  
19       17.22 g of Na<sub>2</sub>(S<sub>2</sub>O<sub>3</sub>) were added and the volume was brought up to 200 ml with  
20       deionized water.
- 21       2) Ag(CN)<sub>2</sub><sup>-</sup> - Equal volumes of 12.5 g/L AgCN and 50g/L KCN were mixed.
- 22       3) Ag(protein) - Two silver protein samples were tested. Silver protein powder (0.5  
23       g of Sigma S-6767, lot # 121H3437, 20% Ag) were added to 10 ml of deionized

1           water. Silver protein powder (1.25 g of Sigma S-9017, lot # 33H3456, 8% Ag)  
2           were added to 10 ml of deionized water.

3       4)  $\text{Ag}(\text{NH}_3)_2^+$ . Silver nitrate was added to ammonium hydroxide to form a black  
4           precipitate. To this solution was added dropwise additional ammonium hydroxide  
5           until the precipitate redissolved, leaving the complex silver ion  $\text{Ag}(\text{NH}_3)_2^+$  in  
6           solution.

7           Also prepared were control solutions containing the same concentrations of  
8           nitrate, ammonia, cyanide and thiosulphate as was present in the test solutions. The anti-  
9           microbial effect of the test solutions was tested by a zone of inhibition test. A sensi disc  
10          (cellulose, 6mm diameter) containing 25 microlitres of each of the test solutions was  
11          placed in the middle of a MHA (Difco media) plate. The silver complexes or ions in the  
12          sensi disc were allowed to diffuse for 4 hours on the MHA plate stored in a 37°C  
13          incubator. After 4 hours, the sensi disc was removed from the plate and analyzed for  
14          silver content using neutron activation analysis (NAA, University of Alberta Slowpoke  
15          Reactor Facility). A further set of plates were used to measure zones of inhibition against  
16          *S. aureus* for each of the silver complexes or ions in the sensi discs. Samples of the agar  
17          were taken from the plates from two locations - the edge of the zone of inhibition and  
18          underneath the discs. The agar samples were analyzed for silver content by NAA. The  
19          control solutions were tested for anti-microbial effect and were found to cause no zone of  
20          inhibition. The results are set forth in Table 10.

**Table 10**  
**Anti-Microbial Effect of Ag<sup>+</sup> Ion Compared to Silver Complex Ions**

Test Solution	ZOI (mm)	Silver Content (ppm)		Edge of ZOI
		In Disc	Under Disc	
Ag(NO <sub>3</sub> )	6	9000	100	1.8
Ag(NH <sub>3</sub> ) <sub>2</sub> <sup>+</sup>	18	7300	221	1.7
Ag(CN) <sub>2</sub> <sup>-</sup>	70	1400	420	4.3
Ag(S <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> <sup>3-</sup>	36	*	*	*
Ag(protein)	6	*	*	*

The above results indicate that silver salts or compounds known to dissociate to produce the  $\text{Ag}^+$  ion (ex. silver nitrate and silver proteins) have a limited anti-microbial effect (6mm ZOI). The anti-microbial effect is greater for silver compositions which release silver complex ions other than  $\text{Ag}^+$  (ex.  $\text{Ag}(\text{NH}_3)_2^+$ ,  $\text{Ag}(\text{CN})_2^-$  and  $\text{Ag}(\text{S}_2\text{O}_3)_2^{2-}$ ). It is also apparent that the silver complex ions are able to diffuse further in the agar medium than the  $\text{Ag}^+$  ion, thereby achieving an anti-microbial effect further from the silver source.

Without being bound by the same, it is believed that the  $\text{Ag}^+$  ion is less efficacious in its anti-microbial effect because it readily precipitates in the agar medium with chloride ions known to be present. The silver complex ions on the other hand demonstrate a higher level of anti-microbial effect and more rapid diffusion. The silver complex ions are also believed not to precipitate with chloride ions to such an extent, making them more suitable for use in industrial systems or with medical devices and the like which come into contact with fluids containing chloride ions.

1      Example 19

2            This example provides comparative diffusion data and zone of inhibition  
3          data for several silver anti-microbial coatings.

4            Three silver films were sputtered under the conditions set forth in Table 11.

5            TABLE 11

Sputtering Conditions	Film 1	Film 2	Film 3
Target (20.3 cm dia)	99.99% Ag	99.99% Ag	99.99% Ag
Working Gas	99/1 wt% Ar/O <sub>2</sub>	99/1 wt% Ar/O <sub>2</sub>	99/1 wt%
Working Gas Pressure	0.7 Pa	5.3 Pa	5.3 Pa
Total Mass Flow	700 sccm	700 sccm	700 sccm
Power	0.5 kW	0.5 kW	0.05 kW
Substrate Temperature	21°C	21°C	21°C
Anode/Cathode Distance	100 mm	100 mm	100 mm

14           The coatings were tested for anti-microbial activity by a ZOI test, as set  
15          forth in previous examples. Silver content was measured by NAA after 4 hours diffusion  
16          in the agar medium, as set forth in Example 18. The comparative results are set out in  
17          Table 12.

18           Table 12  
19          Anti-Microbial Effect of Silver Coatings

Test Film	Ag Species	CZOI (mm)	Under Film	Silver Content (ppm) Edge of ZOI
Film 1	Ag <sup>+</sup>	2	35	0.8
Film 2	AgX <sup>1</sup>	12	8.5	0.7
Film 3	Ag <sup>+</sup> + AgX <sup>1</sup>	12	654	0.4

26          <sup>1</sup> AgX is a silver complex ion or ion pair.

27          For Film 1, which releases predominantly Ag<sup>+</sup> ions, a small ZOI is produced, with the  
28          silver being precipitated as AgCl below the film. For Film 2, a much larger ZOI (6X) is

1 produced with  $\frac{1}{4}$  the amount of silver being precipitated under the wafer. This suggests  
2 that a silver complex ion different than  $\text{Ag}^+$  is formed which diffuses more readily. It is  
3 believed that the diffusion is accelerated as a result of the nature of the complex silver  
4 species. Film 3 releases much more silver than Films 1 or 2, but the bulk of the silver is  
5 in the form of  $\text{Ag}^+$  which precipitates as  $\text{AgCl}$  under the film. However, the size of the  
6 ZOI indicates that, in addition to  $\text{Ag}^+$ , a complex silver ion with much greater mobility  
7 than  $\text{Ag}^+$  is generated. It is believed that one or more of the negative silver hydroxyl ions  
8  $\text{Ag}(\text{OH})_2^-$ ,  $\text{Ag}_2(\text{OH})_3^-$ , or  $\text{Ag}_3(\text{OH})_4^-$  are generated. In that chloride is in the agar medium,  
9 negative silver hydroxyl-chloro complexes may form.

10 Example 20

11       This example is included to demonstrate the preparation of complex ions of  
12 silver cyanide, and the anti-microbial effect of such ions.

13       A silver cyanide bath typically used in electroplating was tested for anti-  
14 microbial effect using 25 microlitres of bath on a sensi disc in a standard ZOI test. The  
15 silver cyanide bath contained 37 g/L silver cyanide, 45 g/L potassium cyanide and 30 g/L  
16 potassium carbonate. The resulting ZOI covered the entire plate, indicating a corrected  
17 ZOI greater than 94 mm. The maximum amount of silver that was available in the AgCN  
18 bath was 30,000 ppm. From previous work it is known that this concentration as  $\text{AgNO}_3$   
19 would not yield a ZOI greater than 6 mm. The effect of the cyanide ion alone was  
20 determined by placing 25 microlitres of 45 g/L KCN on a sensi disc and repeating the ZOI  
21 test. A corrected ZOI of 12.5 mm was produced. A solution of AgCN in distilled water  
22 (37 g/L) was similarly tested for a ZOI. A corrected ZOI of 14 mm was observed.

1           The molar ratio of silver ion to cyanide ion in the bath 0.37:1. This favours  
2           the formation of a negative silver cyanide complex  $\text{Ag}(\text{CN})_2^-$  or  $\text{AgCN}(\text{aq})$  as an ion pair.  
3           The above results demonstrate that these complex silver ions have anti-microbial efficacy  
4           and increased mobility within an agar medium.

5           Thin strips of filter paper were treated with 50 microlitres of either a silver  
6           nitrate solution (10,000 ppm Ag) or a potassium cyanide solution (6,400 ppm CN). The  
7           strips were subjected to a standard ZOI test on the MHA plate. Silver nitrate control strips  
8           gave a corrected ZOI of 8 mm, while the KCN control strips gave no ZOI. When one of  
9           each of the silver nitrate and potassium cyanide strips were placed on the MHA plate at  
10          right angles to each other, the corrected ZOI was 30 mm from the silver nitrate strip and  
11          22 mm from the potassium cyanide strip.

12           This result demonstrates that a complex silver ion resulting from the  
13          combination of silver nitrate and potassium cyanide in the media has greater anti-microbial  
14          efficacy than either solution alone.

15          Example 21

16           This example is included to demonstrate the anti-microbial efficacy of a  
17          complex silver ion of silver chloride.

18           Silver chloride was pressed into a 0.2 g pellet at 413,550 kPa (60,000 psi)  
19          and tested using a standard ZOI test on MHA plates. An 8 mm zone resulted. A mixture  
20          of 0.15 g  $\text{AgCl}$  and 0.05 g  $\text{NaCl}$  pressed into a pellet at 60,000 psi and similarly tested.

21          A 24 mm zone was observed.

1                   The increased concentration of the available chloride ion favours the  
2 formation of the complex silver ion  $\text{AgCl}_2^-$ , which is demonstrated above to have improved  
3 anti-microbial efficacy over  $\text{AgCl}$ .

4                   A silver nitrate solution (10,000 ppm Ag) was tested with sensi discs (25  
5 microlitres) in a ZOI test. A 6 mm zone was observed. The same concentration of  
6  $\text{AgNO}_3$  was tested on an agar plate which had been supplemented with 5% NaCl. A 20  
7 mm zone was observed, indicating improved anti-microbial efficacy. A control plate of  
8 agar supplemented with 5% NaCl did not inhibit bacterial growth (*S. aureus*).

9                   It is believed that the higher concentrations of the chloride ion favoured the  
10 formation of the complex silver ion  $\text{Ag}(\text{Cl})_2^-$ . This species shows three times the anti-  
11 microbial efficacy of  $\text{Ag}^+$  from silver nitrate.

12                  Example 22 - Animal Testing - Irritation

13                  A primary skin irritation study was performed on New Zealand White  
14 (NZW) rabbits using gauze coated with an anti-microbial metal of this invention. The  
15 coating was deposited on a USP type VII gauze using the process conditions of example  
16 7 where the working gas was 99/1 wt% Ar/O<sub>2</sub>.

17                  The coated gauze was placed on abraded and unabraded skin on the side of  
18 a New Zealand White rabbit. At 24 h the gauze was removed and the site was graded for  
19 erythema and edema at 1, 24 and 48 hours after removal.

20                  All animals survived to the end of the study. No erythema, edema or  
21 infection was observed on any animal. It was concluded that the gauze did not produce  
22 local irritation when placed on the skin of male or female NZW rabbits.

1        Example 23 - Animal Testing - Sensitivity

2              The sensitivity of Hartley Guinea Pigs to USP type VII gauze coated with  
3        an anti-microbial metal coating of the present invention was investigated. The gauze was  
4        coated as per Example 7 using 99/1 wt% Ar/O<sub>2</sub>. The split adjuvant technique was used  
5        since the test material was not injectable and the application of dry ice to the induction  
6        area most closely simulates the clinical situation.

7              There was no evidence that the coated gauze induced erythema or edema  
8        and no infection was observed in any of the animals. All animals survived the study.

9              Application of the coated gauze to the skin of male Hartley Guinea Pigs did  
10       not result in local sensitivity when tested by the split adjuvant technique.

11        Example 24

12              This example is included to demonstrate that silver powder/NaCl mixtures  
13        produce an anti-microbial effect from complex silver ions believed to be AgCl<sub>2</sub>.

14              Pellets of silver powder (1 micron) and NaCl (25%) were pressed at the  
15        conditions set out below. The anti-microbial effect was measured by a zone of inhibition  
16        test with the pellets. A comparative control of pressed silver powder was also tested for  
17        a zone of inhibition. The results are shown in Table 13:

18              Table 13  
19        Anti-Microbial Effect of Silver Powder/NaCl

20        Pellet	21        Compression kg (lb.)	ZOI
22        Ag + 25% NaCl	454 (1000)	26 mm
23        Ag + 25% NaCl	1361 (3000)	20 mm
24        Ag + 25% NaCl	2268 (5000)	19 mm
25        Ag powder	454 (1000)	<1 mm

1      Example 25

2            This example illustrates the structural and chemical characteristics of sputter  
3            deposited silver films that exhibit good anti-microbial activity (corrected zone of inhibition,  
4            CZOI) using the zone of inhibition test as set forth in previous examples. The films were  
5            produced by sputtering of a solid 20.3 cm dia planar silver magnetron target onto silicon  
6            wafer substrates (100 mm from the target) under the conditions summarized in Table 14.  
7            The total mass gas flow was 700 sccm. The ratio of substrate temperature to melting point  
8            of silver (1234K),  $T/T_m$ , was less than 0.3, the thickness of the film was nominally 3000Å  
9            and the angle of incidence in each case was 90° (normal incidence). The characteristics  
10          of as deposited silver as well as those that were subsequently annealed (in air at 140°C for  
11          90 minutes) are described in this example. The films were characterized in terms of  
12          structural (grain size, type of defects, recrystallization) and chemical properties (dopant  
13          concentration (wherein dopant refers to atomic %O or oxide content), and electrochemical  
14          rest potential). The results are summarized in Tables 15 and 16.

15            The dopant concentration in the film was measured using x-ray  
16          photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS). In the  
17          XPS technique a monochromatized Al K $\alpha$  x-ray beam was used as the incident beam. A  
18          4kV Ar ion beam was rastered over a 2 mm x 2 mm area in order to remove surface  
19          contaminants and expose a fresh surface for XPS analysis. A positive cesium ion beam  
20          at 12.5 kV was employed for the SIMS analysis. The dopant concentration computed from  
21          XPS and SIMS data is summarized in Tables 15 and 16 for both as deposited and annealed  
22          films. It can be seen that one preferred characteristic of biologically active silver films in  
23          accordance with the invention is the presence of a dopant. The XPS and SIMS data  
24          further showed that the dopant, which in the present case was oxygen or both silver oxide

1 and oxygen, was not chemically bound to the silver atoms in the bulk film. Moreover, the  
2 dopant as oxygen was incorporated in such amounts as to exceed the room temperature  
3 solid solubility in silver.

4 The grain size of as deposited and annealed films was measured from  
5 images taken with a transmission electron microscope (TEM). These data, reported in  
6 Tables 10 and 11, demonstrate that anti-microbial active silver films of this invention have  
7 an average grain size smaller than 200 nm. Active films, as deposited, had an average  
8 grain size less than about 140 nm. The most active films, as deposited, had an average  
9 grain size less than 90 nm. In addition, high resolution transmission electron microscopy  
10 showed that the onset of recrystallization ( $T_{rec}$ ) commenced at about 90°C. Grain growth  
11 of these fine grained, biologically active films, occurred at temperatures well below 0.33  
12  $T_m$ , where  $T_m$  is the melting point of silver in degrees K, in particular below 140°C. In  
13 general, recrystallization diminished anti-microbial activity. However, coatings with higher  
14 levels of silver oxide (coatings 3 and 6) retained anti-microbial activity after annealing.  
15 It is believed that the oxide pins sufficient atomic defects so as to retain anti-microbial  
16 activity after annealing.

17 The TEM analysis further indicated that biologically active silver films  
18 contained a number of growth twins. Upon annealing in air at 140°C for 90 minutes these  
19 growth twins disappeared and annealing twins appeared. These latter twins were, however,  
20 the result of recovery, recrystallization and grain growth which transformed the silver film  
21 into a lower energy state. Evidently, these deposited silver films, along with the associated  
22 growth twins that underwent such grain growth, were in a higher energy state. Thus, the  
23 presence of these aforementioned defects in the as deposited films is a distinguishing  
24 characteristic of anti-microbial coatings in accordance with this invention. Figures 1 and

1 2 are TEM micrographs showing the grain sizes and twins observed in as deposited and  
2 annealed silver films respectively.

The rest potential of the silver films was measured in one molar (1M) potassium hydroxide (KOH) solution using a saturated calomel electrode (SCE) as the reference electrode. Tables 15 and 16 show that the silver films exhibited anti-microbial behaviour only when the rest potential was positive. No biological activity was observed when the rest potential was negative.

Table 14

ID Number	GROWTH CONDITIONS		
	Gas Composition	Pressure Pa (mTorr)	Power(kW)
1	99% Ar, 1% O	1.3 (10)	0.10
2	99% Ar, 1% O	1.3 (10)	0.50
3	99% Ar, 1% O	5.3 (40)	0.05
4	99% Ar, 1% O	5.3 (40)	0.10
5	99% Ar, 1% O	5.3 (40)	0.50
6	80% Ar, 20% O	5.3 (40)	0.10

Table 15  
Structural Characteristics of Sputter Deposited Silver Anti-microbial Coatings

Growth Condition ID Number	As Deposited				C 2 O
	Grain Size	Dopant	Rest Potential	Defects	
	(nm)	Concentration Atomic %O	mV (vs SCE) <sup>1</sup>		(mm)
1	37	5.5	+125	Growth twins	9
2	148	0	-342	.	2
3	21	20.0 <sup>a</sup>	+150	Growth twins	10
4	19	8.0	+135	Growth twins	7
5	41	3.4	+131	Growth twins	9
6	22	58.0 <sup>a</sup>	+146	.	8
Bulk Silver	>200	0	-170	.	<1

- as  $\text{Ag}_2\text{O}$

<sup>1</sup> These values are subject to variability of  $\pm 20$  mV

### - Dot measured

Table 16  
Structural Characteristics of Annealed Silver Anti-microbial Coatings

ID Number	Growth Condition	Annealed at 140°C, 90 Minutes				CZOI
		Grain Size (nm)	Dopant Concentration atomic %O	Rest Potential mV (vs SCE) <sup>1</sup>	Defects	
1		91	-	-6	Annealing twins	1
2		135	0	+224	Annealing twins	0
3		130	16.0 <sup>o</sup>	+121	Annealing twins	10
4		73	0.8	+33	Annealing twins	8
5		132	0.7	-29	Annealing twins	0
6		-	31.0 <sup>o</sup>	+127	-	8
Bulk Silver		>200	0	-170	-	<1

• 44 Ar. 0

<sup>1</sup> These values are subject to variability of  $\pm 20$  mV

9 - not measured

All publications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The terms and expressions in this specification are used as terms of description and not of limitation. There is no intention, in using such terms and expressions, of excluding equivalents of the features illustrated and described, it being recognized that the scope of the invention is defined and limited only by the claims which follow.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS.

1. A method of producing an anti-microbial effect in an alcohol or water based electrolyte, comprising:

5

(a) preparing a silver material such that it forms complex silver ions other than  $\text{Ag}^+$ ,  $\text{Ag}^{2+}$ ,  $\text{Ag}^{3+}$ ,  $\text{Ag(OH)}_2^-$ ,  $\text{Ag}_2(\text{OH})_3^-$ , or  $\text{Ag}_3(\text{OH})_4^-$ , in an amount so as to produce an anti-microbial effect in contact with an alcohol or a water-based electrolyte that is greater than that produced by an equivalent amount of silver as  $\text{Ag}^+$ ; and

10

(b) bringing the silver material into contact with the alcohol or electrolyte to be treated so as to cause the release of the complex silver ions.

15

2. The method as set forth in claim 1, wherein the silver material forms one or more of the complex silver ions  $\text{Ag(CN)}_2^-$ ,  $\text{AgCN}_{(\text{aq})}$ (ion pair),  $\text{Ag(NH}_3)_2^+$ ,  $\text{AgCl}_2^-$ , and  $\text{Ag(S}_2\text{O}_3)_2^{3-}$  in contact with an alcohol or a water based electrolyte.

20  
3. The method as set forth in claim 2, wherein the silver material is prepared as a powder, solution or suspension containing one or more of the complex silver ions.

4. The method as set forth in claim 1, wherein the silver material is a fine grain or nanocrystalline powder.

25  
5. The method as set forth in claim 1, 2, or 3, wherein the silver material is prepared as an anti-microbial coating on a medical device.

6. The method as set forth in claim 1, 2, or 3, wherein the silver material is prepared as a powder for use in the preparation of a topical anti-microbial composition.

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7. The method as set forth in claim 1, wherein the silver material forms one or more of the complex silver ions  $\text{Ag}(\text{CN})_2^-$ ,  $\text{Ag}\text{CN}_{(\text{aq})}$ (ion pair), and  $\text{Ag}(\text{NH}_3)_2^+$  in contact with the alcohol or electrolyte.

5 Dated on 18th day of January 2001

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Fig. 2.



Fig. 1.



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